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CANINE ~~AND FELINE~~ IMMUNOREGULATORY PROTEINS, ~~NUCLEIC ACID MOLECULES,~~ AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

is a continuation-in-part of

This application ~~claims priority to pending~~ U.S. Patent Application Serial No. 09/322,409, filed May 28, 1999, entitled "CANINE AND FELINE IMMUNOREGULATORY PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"; which claims priority to U.S. Provisional Patent Application Serial No. 60/087,306, filed May 29, 1998, entitled "CANINE INTERLEUKIN-4 AND FLT-3 LIGAND PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF"; each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins and/or inhibitors of such proteins or nucleic acid molecules. The present invention also includes therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors, as well as their use to regulate an immune response in an animal.

BACKGROUND OF THE INVENTION

Regulating immune responses in animals is important in disease management. Immune responses can be regulated by modifying the activity of immunoregulatory molecules and immune cells.

Several immunoregulatory molecules have been found in humans and other mammal species. Interleukin-4, produced by activated type 2 helper cells (T_H2 cells), has a number of functions. These functions include promotion of naive T cells and B cells to differentiate and proliferate. IL-4 promotes T_H2 differentiation and inhibits T_H1 development. FMS-like tyrosine kinase 3, (Flt-3 ligand) stimulates the expansion and mobilization of hematopoetic precursor cell stimulating activity. CD40 is a type I transmembrane protein expressed on antigen presenting cells, such as B lymphocytes, and other types of cells such as endothelial cells, epithelial cells, and fibroblasts. CD40 ligand (also known as CD154) is a type II transmembrane protein that is preferentially expressed on activated T lymphocytes. The CD40-CD154 interaction regulates diverse pathways of the immune system, including B cell proliferation, immunoglobulin production and class switching by B cells, activation and clonal expansion of T cells, activity of antigen presenting cells, growth and differentiation of epithelial cells, and regulation of inflammatory responses at mucosal and cutaneous sites. Interleukin-5 is produced by activated type 2 helper cells (T_H2), mast cells, and eosinophils. Its main functions include promotion of growth and differentiation of eosinophils and generation of cytotoxic T cells from thymocytes. Interleukin-13 is produced by T_H1 and T_H2 cells, and promotes growth and differentiation of B cells, up-regulation of MHC class II and CD23 expression on monocytes/macrophages and B cells; and inhibition of production of inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12, among others. Interferon alpha is an antiviral protein that has three major functions: it inhibits viral replication by activating cellular genes that destroy mRNA and inhibit protein translation, it induces MHC class I expression in non virally-infected cells, increasing resistance to

NK cells, and can activate NK cells. GM-CSF, (granulocyte-macrophage colony-stimulating factor) stimulates the production of granulocytes and macrophages.

Prior investigators have disclosed sequences encoding feline IL-4 (Lerner et al., Genbank Accession No. U39634); porcine IL-4 (Zhou et al., Genbank Accession No. L12991); bovine IL-4 (Heussler, V.T., et al., *Gene.* vol. 114, pp. 273-278, 1992); ovine IL-4 (Seow, H.-F., et al., *Gene,* vol. 124, pp. 291-293, 1993); human IL-4 (Yokota, T., et al., *Proc. Natl. Acad. Sci. U.S.A.*, vol. 83(16), pp. 5894-5898, 1986); and murine IL-4 (Sideras, P., et al., *Adv. Exp. Med. Biol.*, vol. 213, pp. 227-236, 1987). Prior investigators have disclosed sequences encoding murine Flt-3 ligand (McClanahan et al., Genbank Accession No. U44024); and human Flt-3 ligand (Lyman et al., *Blood*, vol. 83, pp. 2795-2801, 1994). Prior investigators have disclosed sequences encoding human CD40 (Stamenkovic et al., *EMBO J.,* vol. 8:1403-1410, 1989, GenBank Accession No. (X60592), bovine CD40 (Hirano et al., *Immunology,* vol. 90, pp. 294-300, 1997, GenBank Accession No. U57745), and murine CD40 (Grimaldi et al., *J. Immunol.,* vol. 143, pp.3921-3926. 1992; Torres and Clark, *J. Immunol.,* vol. 148, pp. 620-626, 1992, GenBank Accession No. M83312). Prior investigators have disclosed sequences encoding human CD154 (Graf et al., *Eur. J. Immunol.,* vol. 22, pp. 3191-3194, 1992; Hollenbaugh, et al., *EMBO J.,* vol. 11:4313-4321, 1992; Gauchat et al., *FEBS lett.,* vol., 315, pp. 259-266, 1993; GenBank Accession Nos L07414, X68550, Z15017, X67878, respectively); bovine CD154 (Mertens et al., *Immunogenetics,* vol. 42, pp. 430-431, GenBank Accession No. Z48468); and murine CD154 (Armitage et al., *Nature,* vol. 357, pp. 80-82; 1992, GenBank Accession No. X65453). Prior investigators have disclosed sequences encoding feline interleukin-5 (Padrid et al., *Am. J. Vet. Res.,* vol. 59, pp. 1263-

1269, 1998, GenBank Accession No. AF025436) and human interleukin-5 (Azuma et al., *Nucleic Acids Res.,* vol. 14, pp. 9149-9158, 1986, GenBank Accession No. X04688). Prior investigators have disclosed sequences encoding human interleukin-13 (McKenzie et al., *Proc. Natl Acad. Sci. USA,* vol. 90, pp. 3735-3739, 1993; Minty et al., *Nature,* vol. 362, pp. 248-250, 1993, GenBank Accession Nos L06801 and X69079, respectively); murine interleukin-13 (Brown et al., *J. Immunol.,* vol. 142, pp. 679-687, 1989, GenBank Accession No M23504); and rat interleukin-13 (Lakkis et al., *Biochem. Biophys. Res. Commun.,* Vol. 197, pp. 612-618, 1993, GenBank Accession No. L26913). Prior investigators have disclosed sequences encoding feline interferon (Nakamura, N., Sudo, T., Matsuda, S., Yanai, A., *Biosci. Biotechnol. Biochem.* (1992)Vol: 56 pp 211-214, GenBank accession # E02521). Prior investigators have also disclosed sequences encoding feline GM-CSF (direct submission to GenBank, Accession No. AF053007)

There remains a need for compounds and methods to regulate an immune response by manipulation of the function of canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF.

SUMMARY OF THE INVENTION

The present invention relates to canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins and/or inhibitors of such proteins or nucleic acid molecules. Identification of the nucleic acid molecules of the present invention is unexpected because initial attempts to obtain nucleic acid

5

molecules using PCR were unsuccessful. After numerous attempts, the inventors discovered specific primers that were useful for isolating such nucleic acid molecules.

One embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, and/or SEQ ID NO:21 or a homolog thereof, wherein said homolog has an at least about 50 contiguous nucleotide region identical in sequence to a 50 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, and/or SEQ ID NO:21; (b) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, and/or SEQ ID NO:37 or a homolog thereof, wherein said homolog has an at least 40 contiguous nucleotide region identical in sequence to a 40 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, and/or SEQ ID NO:37; (c) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, and/or SEQ ID NO:50, and/or a

homolog thereof, wherein said homolog has an at least 30 contiguous nucleotide region identical in sequence to a 30 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, and/or SEQ ID NO:50; (d) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and/or SEQ ID NO:59, and/or a homolog thereof, wherein said homolog has an at least 40 contiguous nucleotide region identical in sequence to a 40 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and/or SEQ ID NO:59; (e) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:60 and/or SEQ ID NO:62, and/or a homolog thereof, wherein said homolog has an at least 30 contiguous nucleotide region identical in sequence to a 30 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:60 and/or SEQ ID NO:62; (f) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69 and/or SEQ ID NO:71, and/or a homolog thereof, wherein said homolog has an at least 45 contiguous nucleotide region identical in sequence to a 45 nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID

NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69 and/or SEQ ID NO:71; (g) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and/or SEQ ID NO:79, and/or a homolog thereof, wherein said homolog has an at least 35 contiguous nucleotide region identical in sequence to a 35 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and/or SEQ ID NO:79; (h) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and/or SEQ ID NO:87, and/or a homolog thereof, wherein said homolog has an at least 45 contiguous nucleotide region identical in sequence to a 45 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and/or SEQ ID NO:87; (i) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and/or SEQ ID NO:106, and/or a homolog thereof, wherein said homolog has an at least 15 contiguous nucleotide region identical to a 15 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93,

SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and/or SEQ ID NO:106; (j) an isolated nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:170, and SEQ ID NO:172; and/or (k) an isolated nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:126.

Another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) a nucleic acid molecule having a nucleic acid sequence that is at least about 92 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, and/or SEQ ID NO:21; (b) a nucleic acid molecule having a nucleic acid sequence that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, and/or SEQ ID NO:37; (c) a nucleic acid molecule having a nucleic acid sequence that is at least about 75 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43,

SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, and/or SEQ ID NO:50; (d) a nucleic acid molecule having a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and/or SEQ ID NO:59; (e) a nucleic acid molecule having a nucleic acid sequence that is at least about 70 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:60 and/or SEQ ID NO:62; (f) a nucleic acid molecule having a nucleic acid sequence that is at least about 85 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and/or SEQ ID NO:71; (g) a nucleic acid molecule having a nucleic acid sequence that is at least about 91 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and/or SEQ ID NO:79; (h) a nucleic acid molecule having a nucleic acid sequence that is at least about 90 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and/or SEQ ID NO:87; (i) a nucleic acid molecule having a nucleic acid sequence that is at least about 65 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and/or SEQ ID NO:106; (j) a nucleic acid molecule having a nucleic acid sequence that is

selected from the group consisting of SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:170 and/or SEQ ID NO:172; and/or (k) a nucleic acid molecule having a nucleic acid sequence that is selected from the group consisting of SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, and/or SEQ ID NO:126.

Yet another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) a nucleic acid molecule having a nucleic acid sequence encoding an IL-4 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20 and/or (ii) a protein comprising a fragment of at least 20 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20; (b) a nucleic acid molecule having a nucleic acid sequence encoding a Flt-3 ligand protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34 and/or (ii) a protein comprising a fragment of at least 25 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34; (c) a nucleic

acid molecule having a nucleic acid sequence encoding a Flt-3 ligand protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:44 and/or SEQ ID NO:49 and/or (ii) a protein comprising a fragment of at least 25 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:44 and/or SEQ ID NO:49;

(d) a nucleic acid molecule having a nucleic acid sequence encoding a CD40 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 70 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:53 and/or SEQ ID NO:58 and/or (ii) a protein comprising a fragment of at least 30 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:53 and/or SEQ ID NO:58; (e) a nucleic acid molecule having a nucleic acid sequence encoding a CD40 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 60 percent identical to an amino acid sequence comprising SEQ ID NO:61 and/or (ii) a protein comprising a fragment of at least 20 amino acids of an amino acid sequence comprising SEQ ID NO:61; (f) a nucleic acid molecule having a nucleic acid sequence encoding a CD154 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 80 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:65 and/or SEQ ID NO:70, and/or (ii) a protein comprising a fragment of at least 35 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:65 and/or SEQ ID NO:70; (g) a nucleic acid molecule having a nucleic acid sequence encoding a CD154 protein selected from the

group consisting of (i) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:73 and/or SEQ ID NO:78, and/or (ii) a protein comprising a fragment of at least 50 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:73 and/or SEQ ID NO:78; (h) a nucleic acid molecule having a nucleic acid sequence encoding an IL-5 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86 and/or (ii) a protein comprising a fragment of at least 20 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86; (i) a nucleic acid molecule having a nucleic acid sequence encoding an IL-13 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 70 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105 and/or (ii) a protein comprising a fragment of at least 15 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105; (j) a nucleic acid molecule having a nucleic acid sequence encoding an interferon alpha protein having an amino acid sequence that is selected from the group consisting of amino acid sequence SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:156, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:168, and/or SEQ ID NO:171; (k) a nucleic acid molecule having a nucleic acid sequence encoding a GMCSF protein having an amino acid sequence that is selected from the group consisting of amino acid sequence SEQ ID NO:120, SEQ ID NO:125,

and/or (l) a nucleic acid molecule comprising a complement of any of said nucleic acid molecules as set forth in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), and/or (k), wherein said IL-4 protein elicits an immune response against an IL-4 protein selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20 and/or is a protein with interleukin-4 activity, said Flt-3 ligand protein elicits an immune response against a Flt-3 ligand protein selected from the group consisting of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:44, and/or SEQ ID NO:49 and/or is a protein with Flt-3 ligand activity, said CD40 protein elicits an immune response against a CD40 protein selected from the group consisting of SEQ ID NO:53, SEQ ID NO:58, and/or SEQ ID NO:61 and/or is a protein with CD40 activity, said CD154 protein elicits an immune response against a CD154 protein selected from the group consisting of SEQ ID NO:65, SEQ ID NO:70, SEQ ID NO:73, and/or SEQ ID NO:78 and/or is a protein with CD154 activity, said IL-5 protein elicits an immune response against a IL-5 protein selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86 and/or is a protein with IL-5 activity, said IL-13 protein elicits an immune response against an IL-13 protein selected from the group consisting of SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105 and/or is a protein with IL-13 activity, said interferon alpha protein elicits an immune response against an interferon alpha protein selected from the group consisting of SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:156, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:168, and/or SEQ ID NO:171 and/or is a protein with interferon alpha activity, and/or said GMCSF protein elicits an immune response against a GMCSF protein selected from

the group consisting of SEQ ID NO:120 and/or SEQ ID NO:125 and/or is a protein with GM-CSF activity.

The present invention also includes methods to produce any of the proteins of the present invention using nucleic acid molecules of the present invention and recombinantly using such nucleic acid molecules.

The present invention also includes an isolated protein selected from the group consisting of: (a) (i) an isolated protein of at least about 20 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, and/or SEQ ID NO:19; and/or (ii) an isolated protein of at least about 20 amino acids in length, wherein said protein has an at least 20 contiguous amino acid region identical in sequence to a 20 contiguous amino acid region selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20, wherein said isolated protein elicits an immune response against a canine IL-4 protein and/or has IL-4 activity; (b) (i) an isolated protein of at least about 20 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:33, and/or SEQ ID NO:36; and/or (ii) an isolated protein of at least about 20 amino acids in length, wherein said protein has an at least 20 contiguous amino acid region identical in sequence to a 20 contiguous amino acid region selected from the group consisting of

SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34, wherein said isolated protein is capable of eliciting an immune response against a canine Flt-3 ligand protein and/or has Flt-3 activity; (c) (i) an isolated protein of at least about 20 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:46, and/or SEQ ID NO:48; and/or (ii) an isolated protein of at least about 20 amino acids in length, wherein said protein has an at least 20 contiguous amino acid region identical in sequence to a 20 contiguous amino acid region selected from the group consisting of SEQ ID NO:44 and/or SEQ ID NO:49, wherein said isolated protein is capable of eliciting an immune response against a feline Flt-3 ligand protein and/or has Flt-3 activity; (d)(i) an isolated protein of at least about 30 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least90 contiguous nucleotide region identical in sequence to a 90 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:55, and/or SEQ ID NO:57; and/or (ii) an isolated protein of at least about 30 amino acids in length, wherein said protein has an at least 30 contiguous amino acid region identical in sequence to a 30 contiguous amino acid region selected from the group consisting of SEQ ID NO:53, SEQ ID NO:58, wherein said isolated protein is capable of eliciting an immune response against a canine CD40 protein and/or has CD40 activity; (e) (i) an isolated protein of at least about 20 amino acids in

length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence comprising **SEQ ID NO**:60; and/or (ii) an isolated protein of at least about 20 amino acids in length, wherein said protein has an at least 20 contiguous amino acid region identical in sequence to a 20 contiguous amino acid region comprising the amino acid sequence SEQ ID NO:61, wherein said isolated protein is capable of eliciting an immune response against a feline CD40 protein and/or has CD40 activity; (f)(i) an isolated protein of at least about 35 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least105 contiguous nucleotide region identical in sequence to a 105 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:67, and/or SEQ ID NO:69; and/or (ii) an isolated protein of at least about 35 amino acids in length, wherein said protein has an at least 35 contiguous amino acid region identical in sequence to a 35 contiguous amino acid region selected from the group consisting of SEQ ID NO:65 and/or SEQ ID NO:70, wherein said isolated protein is capable of eliciting an immune response against a canine CD154 protein and/or has CD154 activity; (g)(i) an isolated protein of at least about 50 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least150 contiguous nucleotide region identical in sequence to a 150 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:72, SEQ ID NO:75, and/or SEQ ID NO:77; and/or (ii) an isolated protein of at

least about 50 amino acids in length, wherein said protein has an at least 50 contiguous amino acid region identical in sequence to a 50 contiguous amino acid region selected from the group consisting of SEQ ID NO:73 and/or SEQ ID NO:78, wherein said isolated protein is capable of eliciting an immune response against a feline CD154 protein and/or has CD154 activity; (h)(i) an isolated protein of at least about 20 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least60 contiguous nucleotide region identical in sequence to a 60 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:83, and/or SEQ ID NO:85; and/or (ii) an isolated protein of at least about 20 amino acids in length, wherein said protein has an at least 20 contiguous amino acid region identical in sequence to a 20 contiguous amino acid region selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86, wherein said isolated protein is capable of eliciting an immune response against a canine IL-5 protein and/or has IL-5 activity; (i)(i) an isolated protein of at least about 15 amino acids in length, wherein said protein is encoded by a nucleic acid molecule, wherein said nucleic acid molecule has an at least45 contiguous nucleotide region identical in sequence to a 45 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:99, SEQ ID NO:102, and/or SEQ ID NO:104; and/or (ii) an isolated protein of at least about 15 amino acids in length, wherein said protein has an at least 15 contiguous amino acid region identical in sequence to a 15 contiguous amino acid region selected from the group consisting of SEQ ID NO:92, SEQ

ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105, wherein said isolated protein is capable of eliciting an immune response against a canine IL-13 protein and/or has IL-13 activity; (j) (i) an isolated protein encoded by a nucleic acid molecule selected from the group consisting of SEQ ID NO:107, SEQ ID NO:110, SEQ ID NO:113, SEQ ID NO:116, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167, and/or SEQ ID NO:170, and/or (ii) an isolated protein selected from the group consisting of SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:156, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:168, and/or SEQ ID NO:171, wherein said isolated protein is capable of eliciting an immune response against a feline interferon alpha protein and/or has interferon alpha activity; (k) (i) an isolated protein encoded by a nucleic acid molecule selected from the group consisting of SEQ ID NO:119, SEQ ID NO:122, and/or SEQ ID NO:124, and/or (ii) an isolated protein selected from the group consisting of SEQ ID NO:120 and/or SEQ ID NO:125, wherein said isolated protein is capable of eliciting an immune response against a feline GM-CSF and/or has GM-CSF activity.

The present invention also includes an isolated protein selected from the group consisting of: (a) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20; (b) a protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34; (c) a protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:44

and/or SEQ ID NO:49; (d) a protein having an amino acid sequence that is at least about 70 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:53 and/or SEQ ID NO:58; (e) a protein having an amino acid sequence that is at least about 60 percent identical to an amino acid sequence comprising SEQ ID NO:61; (f) a protein having an amino acid sequence that is at least about 80 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:65 and/or SEQ ID NO:70; (g) a protein having an amino acid sequence that is at least about 85 percent identical to the amino acid sequence SEQ ID NO:73 and/or SEQ ID NO:78; (h) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86; (i) a protein having an amino acid sequence that is at least about 70 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105; (j) a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:156, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:168, and/or SEQ ID NO:171; and/or (k) a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:120, and/or SEQ ID NO:125.

The present invention also includes isolated antibodies that selectively bind to a protein of the present invention.

One aspect of the present invention is a therapeutic composition that, when administered to an animal, regulates an immune response in said animal, said therapeutic composition comprising a therapeutic compound selected from the group consisting of:

20

an immunoregulatory protein of the present invention; a mimetope of any of said immunoregulatory proteins; and a multimeric form of any of said immunoregulatory proteins; an isolated nucleic acid molecule of the present invention; an antibody that selectively binds to any of said immunoregulatory proteins; and/or an inhibitor of a immunoregulatory protein activity identified by its ability to inhibit the activity of any of said immunoregulatory proteins. Yet another aspect of the present invention is a method to regulate an immune response in an animal comprising administering to the animal a therapeutic composition of the present invention.

The present invention also includes a method to produce an immunoregulatory protein, said method comprising culturing a cell capable of expressing said protein, said protein being encoded by a nucleic acid molecule of the present invention.

One embodiment of the present invention is a method to identify a compound capable of regulating an immune response in an animal, said method comprising: (a) contacting an isolated canine IL-4 protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has T cell proliferation stimulating activity; and determining if said putative inhibitory compound inhibits said activity; (b) contacting an isolated canine Flt-3 ligand protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has dendritic precursor cell proliferation stimulating activity; and determining if said putative inhibitory compound inhibits said activity; (c) contacting an isolated feline Flt-3 ligand protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has dendritic precursor cell proliferation stimulating

activity; and determining if said putative inhibitory compound inhibits said activity; (d) contacting an isolated canine CD40 protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has CD40 ligand binding activity; and determining if said putative inhibitory compound inhibits said activity; (e) contacting an isolated feline CD40 protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has CD40 ligand binding activity; and determining if said putative inhibitory compound inhibits said activity; (f) contacting an isolated canine CD154 protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has B cell proliferation activity; and determining if said putative inhibitory compound inhibits said activity; (g) contacting an isolated feline CD154 protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has B cell proliferation activity; and determining if said putative inhibitory compound inhibits said activity; (h) contacting an isolated canine IL-5 protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has TF-1 cell proliferation activity; and determining if said putative inhibitory compound inhibits said activity; (i) contacting an isolated canine IL-13 protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has TF-1 cell proliferation activity; and determining if said putative inhibitory compound inhibits said activity; (j) contacting an isolated feline IFNα protein of the present invention with a putative inhibitory compound under conditions in which, in the absence

of said compound, said protein has inhibition of proliferation of GM-CSF stimulated TF-1 cell activity; and determining if said putative inhibitory compound inhibits said activity; or (k) contacting an isolated feline GMCSF protein of the present invention with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has TF-1 cell proliferation activity; and determining if said putative inhibitory compound inhibits said activity.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF proteins, isolated canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecules, antibodies directed against canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF proteins, and compounds derived therefrom that regulate the immune response of an animal (e.g. inhibitors, antibodies and peptides).

Canine IL-4 protein can refer to a canine IL-4 protein, including homologs thereof. Canine Flt-3 ligand protein can refer to a canine Flt-3 ligand, including homologs thereof, and feline Flt-3 ligand can refer to feline Flt-3 ligand, including homologs thereof. Canine CD40 can refer to a canine CD40, including homologs thereof; feline CD40 can refer to a feline CD40, including homologs thereof. Canine CD154 can refer to a canine CD154, including homologs thereof; feline CD154 can refer to a feline

CD154, including homologs thereof. Canine IL-5 can refer to canine IL-5, including homologs thereof; canine IL-13 can refer to canine IL-13, including homologs thereof. Feline IFNα can refer to a feline IFNα, including homologs thereof, and feline GM-CSF can refer to a feline GM-CSF, including homologs thereof. As used herein, the phrase "regulate an immune response" refers to modulating the activity of cells or molecules involved in an immune response. The term "regulate" can refer to increasing or decreasing an immune response. Regulation of an immune response can be determined using methods known in the art as well as methods disclosed herein. The term, "immunoregulatory protein" refers to a protein that can modulate the activity of cells or of molecules involved in an immune response. An immunoregulatory protein of the present invention refers to a canine IL-4, a canine and/or feline CD40, a canine and/or feline Flt3 ligand, a canine and/or feline CD154, a canine IL-5, a canine IL-13, a feline IFNα, and/or a feline GM-CSF protein as described herein. As used herein, the terms isolated canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF proteins and/or isolated canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecules refer to canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF proteins and/or canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecules derived from mammals and,

as such, can be obtained from their natural source, or can be produced using, for example, recombinant nucleic acid technology or chemical synthesis. Also included in the present invention is the use of these proteins, nucleic acid molecules, antibodies, and/or compounds derived therefrom as therapeutic compositions to regulate the immune response of an animal as well as in other applications, such as those disclosed below.

One embodiment of the present invention is an isolated protein that includes a canine IL-4 protein, a canine and/or feline Flt-3 ligand protein, a canine and/or feline CD40 protein, a canine and/or feline CD154 protein, a canine interleukin-5 protein, a canine interleukin-13 protein, a feline interferon alpha protein, and/or a feline GM-CSF protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein, is a protein that has been removed from its natural milieu. As such, "isolated" and/or "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis. Nucleic acid molecules of the present invention of known length isolated from *Canis familiaris* are denoted as follows: IL-4 is denoted as nCaIL-4_x, for example, nCaIL-4_{549}, wherein "#" refers to the number of nucleotides in that molecule; and in a similar fashion, Flt-3 ligand nucleic acid molecules are referred to as nCaFlt3L_x; CD40, nCaCD40_x; CD154, nCaCD154_x; IL-5, nCaIL-5_x; and

IL-13, nCaIL-13_x. In a similar fashion, Flt-3 ligand nucleic acid molecules of the present invention of known length isolated from *Felis catus* are denoted as nFeFlt3L_x, CD40, nFeCD40_x; CD154, nFeCD154_x; IFNα, nFeIFNα_x; and GM-CSF (also denoted GMCSF), nFeGM-CSF_x. Similarly, proteins of the present invention of known length isolated from *Felis catus* are denoted as PFeFlt$3l_x$, PFeCD40_x, PFeCD154_x, PFeIFNα_x, and/or PFeGM-CSF_x; and proteins of the present invention of known length isolated from *Canis familiaris* are denoted PCaIL-4_x, PCaFlt$3L_x$, PCaCD40_x, PCaCD154_x, PCaIL-5_x, and/or PCaIL-13_x.

As used herein, an isolated canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, and/or feline GM-CSF ligand protein of the present invention (i.e., an canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein, respectively) can be a full-length protein or any homolog of such a protein. An isolated IL-4 protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response against, (or to) an IL-4 protein, bind to an IL-4 receptor, stimulate B cell differentiation or activation or stimulate production of immunoglobulin by a B cell. An isolated Flt-3 ligand protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response against a Flt-3 ligand protein, bind to Flt-3 receptor or stimulate Flt-3 receptor-bearing hematopoietic stem cells, early hematopoietic progenitor cells or immature lymphocytes. An isolated CD40 protein of the present invention, including a homolog,

can be identified in a straight-forward manner by the protein's ability to elicit an immune response against a CD40 protein, bind to CD154 or stimulate CD154-bearing B cells, T cells, and/or epithelial cells. An isolated CD154 protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response to a CD154 protein, bind to CD40 or stimulate CD40-bearing B cells, T cells, and/or epithelial cells. An isolated IL-5 protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response to an IL-5 protein, bind to an IL-5 receptor, and/or stimulate eosinophils and/or cause thymocytes to produce cytotoxic T cells. An isolated IL-13 protein of the present invention, including a homolog, can be identified in a straight-forward maner by the protein's ability to elicit an immune response to an IL-13 protein, bind to an IL-13 receptor, and/or stimulate B cells, up-regulate expression of MHC class II and/or CD23 on monocytes, macrophages and/or B cells; and/or inhibition of proinflammatory cytokines. An isolated interferon alpha protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response to an interferon alpha protein, bind to an interferon alpha receptor, and/or activate NK cells and/or inhibit viral replication. An isolated GM-CSF protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response to a GM-CSF protein, bind to a GM-CSF receptor, and/or activate granulocytes and/or macrophages. Examples of protein homologs of the present invention include immunoregulatory proteins of the present invention in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide),

inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the protein homolog includes at least one epitope capable of eliciting an immune response against the parent protein, of binding to an antibody directed against the parent protein and/or of binding to the parent's receptor, where the term parent refers to the longer and/or full-length protein that the homolog is derived from. That is, when the homolog is administered to an animal as an immunogen, using techniques known to those skilled in the art, the animal will produce an immune response against at least one epitope of an immunoregulatory protein of the present invention, depending upon which protein is administered to an animal. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art. As used herein, the term "epitope" refers to the smallest portion of a protein capable of selectively binding to the antigen binding site of an antibody. It is well accepted by those skilled in the art that the minimal size of a protein epitope capable of selectively binding to the antigen binding site of an antibody is about five or six to seven amino acids.

Homologs of immunoregulatory proteins of the present invention can be the result of natural allelic variation, including natural mutation. Protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein and/or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

Immunoregulatory proteins of the present invention include variants of a full-length protein of a protein of the present invention. Such variants include proteins that are less than full-length. As used herein, variants of the present invention refer to nucleic acid molecules that are naturally-occurring as defined below, and may result from alternative RNA splicing, alternative termination of an amino acid sequence or DNA recombination. Examples of variants include allelic variants as defined below. It is to be noted that a variant is an example of a homolog of the present invention.

Immunoregulatory proteins of the present invention are encoded by nucleic acid molecules of the present invention. As used herein, an IL-4 nucleic acid molecule includes nucleic acid sequences related to a natural canine IL-4 gene. As used herein, a Flt-3 ligand nucleic acid molecule includes nucleic acid sequences related to a natural canine Flt-3 ligand gene. As used herein, a CD40 nucleic acid molecule includes nucleic acid sequences related to a natural CD40 gene. As used herein, a CD154 nucleic acid molecule includes nucleic acid sequences related to a natural CD154 gene. As used herein, an IL-5 nucleic acid molecule includes nucleic acid sequences related to a natural IL-5 gene. As used herein, an IL-13 nucleic acid molecule includes nucleic acid sequences related to a natural IL-13 gene. As used herein, an IFNα nucleic acid molecule includes nucleic acid sequences related to a natural IFNα gene. As used herein, a GM-CSF nucleic acid molecule includes nucleic acid sequences related to a natural GM-CSF gene. As used herein, a canine IL-4, a canine and/or feline CD40, a canine and/or feline Flt3 ligand, a canine and/or feline CD154, a canine IL-5, a canine IL-13, a feline IFNα, and/or a feline GM-CSF gene refers to the natural genomic elements that encode an canine IL-4, a canine and/or feline CD40, a canine and/or feline Flt3 ligand, a canine

and/or feline CD154, a canine IL-5, a canine IL-13, a feline IFNα, and/or a feline GM-CSF protein, respectively, and includes all regions such as regulatory regions that control production of the protein encoded by the gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a sequence may include that sequence in one contiguous array, or may include the sequence as fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that translates into a protein. A full-length coding region is that region that is translated into a full-length, i.e., a complete, protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment, an IL-4 gene of the present invention includes the nucleic acid sequence SEQ ID NO:1, as well as the complement of SEQ ID NO:1. Nucleic acid sequence SEQ ID NO:1 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule nCaIL-4_{549}, the production of which is disclosed in the Examples. Nucleic acid molecule nCaIL-4_{549} comprises an apparently full-length coding region of canine IL-4. The complement of SEQ ID NO:1 (represented herein by SEQ ID NO:3) refers to the nucleic acid sequence of the strand fully complementary to the strand having SEQ ID NO:1, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is fully complementary to (i.e., can form a double helix with) the strand for which the sequence is cited. It should be noted that since nucleic acid

sequencing technology is not entirely error-free, SEQ ID NO:1 (as well as other nucleic acid and protein sequences presented herein) represents an apparent nucleic acid sequence of the nucleic acid molecule encoding an immunoregulatory protein of the present invention.

In another embodiment, a Flt-3 ligand gene of the present invention includes the nucleic acid sequence SEQ ID NO:6, as well as the complement represented by SEQ ID NO:8. Nucleic acid sequence SEQ ID NO:6 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule $nCaFlt3L_{1013}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nCaFlt3L_{1013}$ comprises an apparently full-length coding region of canine Flt-3 ligand.

In another embodiment, a Flt-3 ligand gene of the present invention includes the nucleic acid sequence SEQ ID NO:43, as well as the complement represented by SEQ ID NO:45. Nucleic acid sequence SEQ ID NO:43 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule $nFeFlt3L_{942}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nFeFlt3L_{942}$ comprises an apparently full-length coding region of feline Flt-3 ligand.

In another embodiment, a CD40 gene of the present invention includes the nucleic acid sequence SEQ ID NO:52, as well as the complement represented by SEQ ID NO:54. Nucleic acid sequence SEQ ID NO:52 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule $nCaCD40_{1425}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nCaCD40_{1425}$ comprises an apparently full-length coding region of canine CD40.

In another embodiment, a CD40 gene of the present invention includes the nucleic acid sequence SEQ ID NO:60, as well as the complement represented by SEQ ID NO:62. Nucleic acid sequence SEQ ID NO:60 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule $nFeCD40_{336}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nFeCD40_{336}$ comprises an apparent portion of the coding region of feline CD40.

In another embodiment, a CD154 gene of the present invention includes the nucleic acid sequence SEQ ID NO:64, as well as the complement represented by SEQ ID NO:66. Nucleic acid sequence SEQ ID NO:64 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule $nCaCD154_{1878}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nCaCD154_{1878}$ comprises an apparently full-length coding region of canine CD154.

In another embodiment, a CD154 gene of the present invention includes the nucleic acid sequence SEQ ID NO:72, as well as the complement represented by SEQ ID NO:74. Nucleic acid sequence SEQ ID NO:72 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule $nFeCD154_{885}$, the production of which is disclosed in the Examples. Nucleic acid molecule $nFeCD154_{885}$ comprises an apparently full-length coding region of feline CD154.

In another embodiment, an IL-5 gene of the present invention includes the nucleic acid sequence SEQ ID NO:80, as well as the complement represented by SEQ ID NO:82. Nucleic acid sequence SEQ ID NO:80 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule $nCaIL\text{-}5_{610}$, the production of

which is disclosed in the Examples. Nucleic acid molecule nCaIL-5_{610} comprises an apparently full-length coding region of canine IL-5.

In another embodiment, an IL-13 gene of the present invention includes the nucleic acid sequence SEQ ID NO:91, as well as the complement represented by SEQ ID NO:93. Nucleic acid sequence SEQ ID NO:91 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaIL-13_{1302}, the production of which is disclosed in the Examples. Nucleic acid molecule nCaIL-13_{1302} comprises an apparently full-length coding region of canine IL-13.

In another embodiment, an IFNα gene of the present invention includes the nucleic acid sequence SEQ ID NO:107, SEQ ID NO:110, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167, or SEQ ID NO:170, as well as the complement represented by, respectively, SEQ ID NO:109, SEQ ID NO:112, SEQ ID NO:157, SEQ ID NO:160, SEQ ID NO:163, or SEQ ID NO:166, SEQ ID NO:169, and SEQ ID NO:172. Nucleic acid sequences SEQ ID NO:107, SEQ ID NO:110, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167, and SEQ ID NO:170 represent the deduced sequences of the coding strands of cDNAs denoted herein as nucleic acid molecules nFeIFNα$_{567a}$, nFeIFNα$_{567b}$, nFeIFNα$_{567c}$, nFeIFNα$_{498c}$, nFeIFNα$_{582d}$, nFeIFNα$_{513d}$, nFeIFNα$_{567e}$, and nFeIFNα$_{498e}$, respectively. Each of these nucleic acid molecules, the production of which is disclosed in the Examples, comprises an apparently full-length coding region of a feline IFNα protein.

In another embodiment, a GM-CSF gene of the present invention includes the nucleic acid sequence SEQ ID NO:119, as well as the complement represented by SEQ

ID NO:121. Nucleic acid sequence SEQ ID NO:119 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeGM-CSF$_{444}$, the production of which is disclosed in the Examples. Nucleic acid molecule nFeGM-CSF$_{444}$ comprises an apparently full-length coding region of feline GM-CSF.

Additional immunoregulatory nucleic acid molecules and proteins of the present invention having specific sequence identifiers are described in Table 1.

Table 1. Sequence identification numbers (SEQ ID NOs) and their corresponding nucleic acid molecules or proteins.

SEQ ID NO:	DESCRIPTION
1	nCaIL-4$_{549}$ coding strand
2	PCaIL-4$_{132}$
3	nCaIL-4$_{549}$ complementary strand
4	nCaIL-4$_{396}$ coding strand
5	nCaIL-4$_{396}$ complementary strand
6	nCaFlt3L$_{1013}$ coding strand
7	PCaFlt3L$_{294}$
8	nCaFlt3L$_{1013}$ complementary strand
9	nCaFlt3L$_{882}$ coding strand
10	nCaFlt3L$_{882}$ complementary strand
19	nCaIL-4$_{324}$ coding strand
20	PCaIL-4$_{108}$
21	nCaIL-4$_{324}$ complementary strand
22	nCaFlt3L$_{804}$ coding strand
23	PCaFlt3L$_{268}$
24	nCaFlt3L$_{804}$ complementary strand
25	nCaFlt3L$_{985}$ coding strand

SEQ ID NO:	DESCRIPTION
26	$PCaFlt3L_{276}$
27	$nCaFlt3L_{985}$ complementary strand
28	$nCaFlt3L_{828}$ coding strand
29	$nCaFlt3L_{828}$ complementary strand
30	$nCaFlt3L_{750}$ coding strand
31	$PCaFlt3L_{250}$
32	$nCaFlt3L_{750}$ complementary strand
33	$nCaFlt3L_{1019}$ coding strand
34	$PCaFlt3L_{31}$
35	$nCaFlt3L_{1019}$ complementary strand
36	$nCaFlt3L_{93}$ coding strand
37	$nCaFlt3L_{93}$ complementary strand
41	$nFeFlt3L_{395}$ coding strand
42	$nFeFlt3L_{793}$ coding strand
43	$nFeFlt3L_{942}$ coding strand
44	$PFeFlt3L_{291}$
45	$nFeFlt3L_{942}$ complementary strand
46	$nFeFlt3L_{873}$ coding strand
47	$nFeFlt3L_{873}$ complementary strand
48	$nFeFlt3L_{795}$ coding strand
49	$PFeFlt3L_{265}$
50	$nFeFlt3L_{795}$ complementary strand
51	$nCaCD40_{321}$ coding strand
52	$nCaCD40_{1425}$ coding strand
53	$PCaCD40_{274}$
54	$nCaCD40_{1425}$ complementary strand

SEQ ID NO:	DESCRIPTION
55	$nCaCD40_{822}$ coding strand
56	$nCaCD40_{822}$ complementary strand
57	$nCaCD40_{765}$ coding strand
58	$PCaCD40_{255}$
59	$nCaCD40_{765}$ complementary strand
60	$nFeCD40_{336}$ coding strand
61	$PFeCD40_{112}$
62	$nFeCD40_{336}$ complementary strand
63	$nCaCD154_{390}$ coding strand
64	$nCaCD154_{1878}$ coding strand
65	$PCaCD154_{260}$
66	$nCaCD154_{1878}$ complementary strand
67	$nCaCD154_{780}$ coding strand
68	$nCaCD154_{780}$ complementary strand
69	$nCaCD154_{633}$ coding strand
70	$PCaCD154_{211}$
71	$nCaCD154_{633}$ complementary strand
72	$nFeCD154_{885}$ coding strand
73	$PFeCD154_{260}$
74	$nFeCD154_{885}$ complementary strand
75	$nFeCD154_{780}$ coding strand
76	$nFeCD154_{780}$ complementary strand
77	$nFeCD154_{633}$ coding strand
78	$PFeCD154_{211}$
79	$nFeCD154_{633}$ complementary strand
80	$nCaIL\text{-}5_{610}$ coding strand

SEQ ID NO:	DESCRIPTION
81	PCaIL-5_{134}
82	nCaIL-5_{610} complementary strand
83	nCaIL-5_{402} coding strand
84	nIL-5_{402} complementary strand
85	nCaIL-5_{345} coding strand
86	PCaIL-5_{115}
87	nCaIL-5_{345} complementary strand
88	nCaIL-13_{166} coding strand
89	nCaIL-13_{272} coding strand
90	nCaIL-13_{278} coding strand
91	nCaIL-13_{1302} coding strand
92	PCaIL-13_{131}
93	nCaIL-13_{1302} complementary strand
94	nCaIL-13_{393} coding strand
95	nCaIL-13_{393} complementary strand
96	nCaIL-13_{333} coding strand
97	PaIL-13_{111}
98	nCaIL-13_{333} complementary strand
99	nCaIL-13_{1269} coding strand
100	PCaIL-13_{130}
101	nCaIL-13_{1269} complementary strand
102	nCaIL-13_{390} coding strand
103	nCaIL-13_{390} complementary strand
104	nCaIL-13_{330} coding strand
105	PCaIL-13_{110}
106	nCaIL-13_{330} complementary strand

SEQ ID NO:	DESCRIPTION
107	$nFeIFN\alpha_{567a}$ coding strand
108	$PFeIFN\alpha_{189a}$
109	$nFeIFN\alpha_{567a}$ complementary strand
110	$nFeIFN\alpha_{567b}$ coding strand
111	$PFeIFN\alpha_{189b}$
112	$nFeIFN\alpha_{567b}$ complementary strand
113	$nFeIFN\alpha_{498a}$ coding strand
114	$PFeIFN\alpha_{166a}$
115	$nFeIFN\alpha_{498a}$ complementary strand
116	$nFeFeIFN\alpha_{498b}$ coding strand
117	$PFeIFN\alpha_{166b}$
118	$nFeIFN\alpha_{498b}$ complementary strand
119	$nFeGMCSF_{444}$ coding strand
120	$PFeGMCSF_{144}$
121	$nFeGMCSF_{444}$ complementary strand
122	$nFeGMCSF_{432}$ coding strand
123	$nFeGMCSF_{432}$ complementary strand
124	$nFeGMCSF_{381}$ coding strand
125	$PFeGMCSF_{127}$
126	$nFeGMCSF_{381}$ complementary strand
155	$nFeIFN\alpha_{567c}$
156	$PFeIFN\alpha_{189c}$
157	$nFeIFN\alpha_{567c}$ complementary strand
158	$nFeIFN\alpha_{498c}$
159	$PFeIFN\alpha_{166c}$
160	$nFeIFN\alpha_{498c}$ complementary strand

SEQ ID NO:	DESCRIPTION
161	$nFeIFN\alpha_{582d}$
162	$PFeIFN\alpha_{194d}$
163	$nFeIFN\alpha_{582d}$ complementary strand
164	$nFeIFN\alpha_{513d}$
165	$PFeIFN\alpha_{171d}$
166	$nFeIFN\alpha_{513d}$ complementary strand
167	$nFeIFN\alpha_{567e}$
168	$PFeIFN\alpha_{189e}$
169	$nFeIFN\alpha_{567e}$ complementary strand
170	$nFeIFN\alpha_{498e}$
171	$PFeIFN\alpha_{166e}$
172	$nFeIFN\alpha_{498e}$ complementary strand

In another embodiment, an IL-4 gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, SEQ ID NO:21, and/or any other IL-4 nucleic acid sequence cited herein. In another embodiment, a Flt-3 ligand gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50 and/or any other Flt-3 ligand nucleic acid sequence cited herein. In another embodiment, a CD40 gene or nucleic acid

molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62 and/or any other CD40 nucleic acid sequence cited herein. In another embodiment, a CD154 gene or nucleic acid moleucle can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:79 and/or any other CD154 nucleic acid sequence cited herein. In another embodiment, an IL-5 gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87 and/or any other IL-5 nucleic acid sequence cited herein. In another embodiment, an IL-13 gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:106 and/or any other IL-13 nucleic acid sequence cited herein. In another embodiment, an IFNα gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:170 and/or SEQ ID NO:172, and/or any other IFNα nucleic acid

sequence cited herein. In another embodiment, a GM-CSF gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, and/or SEQ ID NO:126 and/or any other GM-CSF nucleic acid cited herein. An allelic variant of a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF gene, including the particular SEQ ID NO's cited herein, is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including the particular SEQ ID NO's cited herein, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Also included in the term allelic variant are allelic variants of cDNAs derived from such genes. Because natural selection typically selects against alterations that affect function, allelic variants usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given animal, since the respective genomes are diploid, and sexual reproduction will result in the reassortment of alleles.

The minimal size of an canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein homolog of the present invention is a

size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridize under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, and Meinkoth, *et al.*, 1984, *Anal. Biochem. 138*, 267-284, each of which is incorporated herein by this reference. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents, such as formamide, the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule. As defined in the formula below, T_m is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

$$T_m = 81.5°C + 16.6 \log M + 0.41(\%G + C) - 500/n - 0.61(\%\text{formamide}).$$

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation:

$$T_d = 4(G + C) + 2(A + T).$$

A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base pair mismatch, i.e. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m decreases about 1°C for each 1% of mismatched base pairs for hybrids greater than about 150 bp, and T_d decreases about 5°C for each mismatched base pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions, by altering, for example, the salt concentration, the formamide concentration or the temperature, so that only nucleic acid hybrids with greater than a specified % base pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow about 30% base pair mismatch, i.e., about 70% identity. Because one skilled in the art can easily determine whether a given nucleic acid

molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene or specified nucleic acid molecule under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures. Typically, the actual hybridization reaction is done under non-stringent conditions, i.e., at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

Preferred portions, or fragments, of a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF, protein of the present invention include at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 60 amino acids, at least 75 amino acids or at least 100 amino acids. An IL-4, IL-5, and/or IL-13 protein of the present invention can include at least a portion of an IL-4, IL-5, and/or IL-13 protein that is capable of binding to an IL-4,

IL-5, and/or IL-13 receptor, respectively. IL-4, IL-5, and IL-13 receptors are known to those of skill in the art, and are described in Janeway et al., in *Immunobiology, the Immune System in Health and Disease*, Garland Publishing, Inc., NY, 1996 (which is incorporated herein by this reference in its entirety). The IL-4, IL-5, and/or IL-13 receptor-binding portion of an IL-4, IL-5, and/or IL-13 protein, respectively, can be determined by incubating the protein with an isolated IL-4, IL-5, and/or IL-13 receptor, as appropriate, or a cell having an IL-4, IL-5, and/or IL-13 receptor on its surface, as appropriate. IL-4, IL-5, and/or IL-13 protein binding to purified IL-4, IL-5, and/or IL-13 receptor, respectively, can be determined using methods known in the art including Biacore® screening, confocal immunofluorescent microscopy, immunoprecipitation, gel chromatography, determination of inhibition of binding of antibodies that bind specifically to the IL-4, IL-5, and/or IL-13 binding domain of an IL-4, IL-5, and/or IL-13 receptor, ELISA using an IL-4, IL-5, and/or IL-13 receptor, respectively, labeled with a detectable tag such as an enzyme or chemiluminescent tag or yeast-2 hybrid technology.

A Flt-3 ligand protein of the present invention can include at least a portion of a Flt-3 ligand protein that is capable of binding to Flt-3 receptor or stimulating Flt-3 receptor-bearing hematopoietic stem cells, early hematopoietic progenitor cells or immature lymphocytes. Flt-3 receptors are known to those of skill in the art, and are described in Drexler, *Leukemia*, vol. 10, pp. 588-599, 1996 (which is incorporated herein in its entirety by this reference). The Flt-3 receptor-binding portion of a Flt-3 ligand protein can be determined by incubating the protein with isolated Flt-3 receptor or a cell having a Flt-3 receptor on its surface. Flt-3 ligand protein binding to purified Flt-3 receptor can be determined using methods known in the art including Biacore® screening, confocal

immunofluorescent microscopy, immunoprecipitation, gel chromatography, determination of inhibition of binding of antibodies that bind specifically to the Flt-3 ligand binding domain of a Flt-3 receptor, ELISA using a Flt-3 receptor labeled with a detectable tag such as an enzyme or chemiluminescent tag or yeast-2 hybrid technology. A CD40 and/or CD154 protein of the present invention can include at least a portion of a CD40 and/or CD154 protein that is capable of binding to a CD40 and/or CD154 receptor, respectively, or stimulating CD40 and/or CD154 receptor-bearing hematopoietic stem cells, early hematopoietic progenitor cells or immature lymphocytes. The CD40 and/or CD154 receptor-binding portion of a CD40 and/or CD154 protein can be determined by incubating the protein with isolated CD40 and/or CD154 receptor, as appropriate, or a cell having a CD40 and/or CD154 receptor on its surface, as appropriate. CD40 and/or CD154 protein binding to CD154 and/or CD40, respectively, can be determined using methods known in the art including Biacore® screening, confocal immunofluorescent microscopy, immunoprecipitation, gel chromatography, determination of inhibition of binding of antibodies that bind specifically to the CD40 and/or CD154 binding domain of CD40 and/or CD154, as appropriate, ELISA using a CD40 and/or CD154 labeled with a detectable tag such as an enzyme or chemiluminescent tag or yeast-2 hybrid technology.

The present invention also includes mimetopes of canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF proteins of the present invention. As used herein, a mimetope of an immunoregulatory protein of the present invention refers to any compound that is able to mimic the activity of such a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline

CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein, respectively, often because the mimetope has a structure that mimics the particular protein. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation such as all-D retro peptides; anti-idiotypic and/or catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and/or synthetic or natural organic molecules, including nucleic acids. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner.

One embodiment of an immunoregulatory protein of the present invention is a fusion protein that includes either a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein-containing domain, each attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: link two or more immunoregulatory proteins of the present invention, to form multimeric forms of an immunoregulatory protein of the present invention; enhance a protein's stability; act as an immunopotentiator to enhance an immune response against an canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein; and/or assist in purification of an canine interleukin-4, canine or feline Flt-3 ligand, canine or feline

CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the IL-4-containing domain, or the Flt-3 ligand-containing domain, or the CD40-containing domain, or the CD154-containing domain, or the IL-5-containing domain, or the IL-13-containing domain, or the IFNα-containing domain, or GM-CSF-containing domain, of a protein and can be susceptible to cleavage in order to enable straight-forward recovery of either canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein, respectively. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of an canine interleukin-4-, canine or feline Flt-3 ligand-, canine or feline CD40-, canine or feline CD154-, canine interleukin-5-, canine interleukin-13-, feline interferon alpha-, or feline GM-CSF-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a portion of -galactosidase, a strep tag peptide, a T7 tag peptide, a Flag™ peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More

preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide.

A suitable fusion segment that links one IL-4 protein to another IL-4 protein, or one Flt-3 ligand protein to another Flt-3 ligand protein, or one CD40 protein to another CD40 protein, or one CD154 protein to another CD154 protein, or one IL-5 protein to another IL-5 protein to another IL-5 protein, or one IL-13 protein to another IL-13 protein, or one IFNα protein to another IFNα protein, or one GM-CSF protein to another GM-CSF protein, includes any amino acid sequence that enables such proteins to be linked while maintaining the biological function of either the canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF, proteins, respectively. Selection of a suitable linker is dependent upon how many proteins are to be linked to form one multimeric molecule and from where on either the canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF molecule the linker extends. Preferably, a linker fusion segment of the present invention comprises a peptide of from about 6 amino acid residues to about 40 residues, more preferably from about 6 residues to about 30 residues in length.

In another embodiment, an canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein of the present invention also includes at least one additional protein segment that is capable of targeting either canine

interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein, respectively, to a desired cell or receptive molecule. Such a multivalent targeting protein can be produced by culturing a cell transformed with a nucleic acid molecule comprising two or more nucleic acid domains joined together in such a manner that the resulting nucleic acid molecule is expressed as a multivalent targeting protein containing a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein or portion thereof and/or at least one targeting compound capable of delivering the canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein, respectively, to a desired site in an animal.

Examples of multivalent targeting proteins include, but are not limited to, a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein of the present invention attached to one or more compounds that can bind to a receptive molecule on the surface of a cell located in an area of an animal where regulation of an immune response is desired. One of skill in the art can select appropriate targeting fusion segments depending upon the cell or receptive molecule being targeted.

Another example of a multivalent protein of the present invention includes, but is not limited to, a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon

alpha, or feline GM-CSF protein of the present invention attached to one or more proteins that are potentially antigenic in mammals. Thus, immunogenicity of the potentially antigenic protein could be enhanced by administering to a mammal together with an immunoregulatory protein of the present invention.

A naturally-occurring variant of a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein of the present invention is preferably isolated from (including isolation of the natural protein or production of the protein by recombinant or synthetic techniques) from mammals, including but not limited to dogs (i.e., canids), cats (i.e., felids), horses (i.e., equids), humans, cattle, chinchillas, ferrets, goats, mice, minks, rabbits, raccoons, rats, sheep, squirrels, swine, chickens, ostriches, quail and/or turkeys as well as other furry animals, pets, zoo animals, work animals and/or food animals. Particularly preferred animals from which to isolate canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF proteins are dogs, cats, horses and/or humans.

A preferred isolated protein of the present invention is a protein encoded by at least one of the following nucleic acid molecules:nCaIL-4_{549}, nCaIL-4_{396}, nCaIL-4_{324}, nCaFlt3L$_{1013}$, nCaFlt3L$_{882}$, nCaFlt3L$_{804}$, nCaFlt3L$_{828}$, nCaFlt3L$_{985}$, nCaFlt3L$_{1019}$, nCaFlt3L$_{93}$, nCaFlt3L$_{750}$, nFeFlt3L$_{395}$, nFeFlt3L$_{793}$, nFeFlt3L$_{942}$, nFeFlt3L$_{873}$, nFeFlt3L$_{795}$, nCaCD40$_{321}$, nCaCD40$_{1425}$, nCaCD40$_{822}$, nCaCD40$_{765}$, nFeCD40$_{336}$, nCaCD154$_{390}$, nCaCD1541$_{878}$, nCaCD154$_{780}$, nCaCD154$_{633}$, nFeCD154$_{885}$, nFeCD154$_{780}$, nFeCD154$_{633}$, nCaIL-5_{610}, nCaIL-5_{402}, nCaIL-5_{345}, nCaIL-13_{166}, nCaIL-13_{272}, nCaIL-13_{278}, nCaIL-13_{1302},

nCaIL-13_{393}, nCaIL-13_{333}, nCaIL-13_{1269}, nCaIL-13_{390}, nCaIL-13_{330}, nFeIFNα_{567a}, nFeIFNα_{567b}, nFeIFNα_{567c}, nFeIFNα_{498a}, nFeIFNα_{498b}, nFeIFNα_{498c}, nFeIFNα_{582d}, nFeIFNα_{513d}, nFeIFNα_{567e}, nFeIFNα_{498e}, nFeGMCSF$_{444}$, nFeGMCSF$_{432}$, nFeGMCSF$_{381}$ and/or allelic variants of any of these nucleic acid molecules. Also preferred is an isolated protein that is encoded by a nucleic acid molecule the having nucleic acid sequence SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:99, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:107, SEQ ID NO:110, SEQ ID NO:113, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:161, SEQ ID NO:164, SEQ ID NO:167, and SEQ ID NO:170; and/or an allelic variant of such a nucleic acid molecule.

Translation of SEQ ID NO:1, the coding strand of nCaIL-4_{549}, yields a protein of about 132 amino acids, denoted herein as PCaIL-4_{132}, the amino acid sequence of which is presented in SEQ ID NO:2, assuming an open reading frame having an initiation codon spanning from nucleotide 43 through nucleotide 45 of SEQ ID NO:1 and a stop codon spanning from nucleotide 439 through nucleotide 441 of SEQ ID NO:1.

Translation of SEQ ID NO:6, the coding strand of $nCaFlt3L_{1013}$, yields a protein of about 294 amino acids, denoted herein as $PCaFlt3L_{294}$, the amino acid sequence of which is presented in SEQ ID NO:7, assuming an open reading frame having an initiation codon spanning from nucleotide 35 through nucleotide 37 of SEQ ID NO:6 and a stop codon spanning from nucleotide 917 through nucleotide 919 of SEQ ID NO:6.

Translation of SEQ ID NO:43, the coding strand for $nFeFlt3L_{942}$, yields a protein of about 291 amino acids, denoted herein as $PFeFlt3L_{291}$, the amino acid sequence of which is presented in SEQ ID NO:44, assuming an open reading frame having an initiation codon spanning from nucleotide 31 through nucleotide 33 of SEQ ID NO:43 and a stop codon spanning from nucleotide 904 through nucleotide 906 of SEQ ID NO:43.

Translation of SEQ ID NO:52, the coding strand for $nCaCD40_{1425}$, yields a protein of about 274 amino acids, denoted herein as $PCaCD40_{274}$, the amino acid sequence of which is presented in SEQ ID NO:53, assuming an open reading frame having an initation codon spanning from nucleotide 196 through nucleotide 198 of SEQ ID NO:52 and a stop codon spanning from about nucleotide 1018 through nucleotide 1020 of SEQ ID NO:52.

Translation of SEQ ID NO:60, the coding strand for $nFeCD40_{336}$, yields a protein of about 112 amino acids, denoted herein as $PFeCD40_{112}$, the amino acid sequence of which is presented in SEQ ID NO:61, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:60.

Translation of SEQ ID NO:64, the coding strand for $nCaCD154_{1878}$, yields a protein of about 260 amino acids, denoted herein as $PCaCD154_{260}$, the amino acid

sequence of which is presented in SEQ ID NO:65, assuming an open reading frame having an initiation codon spanning from nucleotide 284 through nucleotide 286 of SEQ ID NO:64 and a stop codon spanning from nucleotide 1064 through nucleotide 1066 of SEQ ID NO:64.

Translation of SEQ ID NO:72, the coding strand for $nFeCD154_{885}$, yields a protein of about 260 amino acids, denoted herein as $PFeCD154_{260}$, the amino acid sequence of which is presented in SEQ ID NO:73, assuming an open reading frame having an initiation codon spanning from nucleotide 29 through nucleotide 31 of SEQ ID NO:72, and a stop codon spanning from nucleotide 809 through nucleotide 811 of SEQ ID NO:72.

Translation of SEQ ID NO:80, the coding strand for $nCaIL\text{-}5_{610}$, yields a protein of about 134 amino acids, denoted herein as $PCaIL\text{-}5_{134}$, the amino acid sequence of which is presented in SEQ ID NO:81, assuming an open reading frame having an initiation codon spanning from nucleotide 29 through nucleotide 31 of SEQ ID NO:80, and a stop codon spanning from nucleotide 431 through nucleotide 433 of SEQ ID NO:80.

Translation of SEQ ID NO:91, the coding strand for $nCaIL\text{-}13_{1302}$, yields a protein of about 131 amino acids, denoted herein as $PCaIL\text{-}13_{131}$, the amino acid sequence of which is presented in SEQ ID NO:92, assuming an open reading frame having an initiation codon spanning from nucleotide 52 through nucleotide 54 of SEQ ID NO:91 and a stop codon spanning from nucleotide 445 through nucleotide 447 of SEQ ID NO:91.

Translation of SEQ ID NO:107, the coding strand for $nFeIFN\alpha_{567a}$, yields a protein of about 189 amino acids, denoted herein as $PFeIFN\alpha_{189a}$, the amino acid sequence of

which is presented in SEQ ID NO:108, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 and a last codon prior to a stop codon spanning from nucleotide 565 through nucleotide 567 of SEQ ID NO:107.

Translation of SEQ ID NO:110, the coding strand for nFeIFNα_{567b}, yields a protein of about 189 amino acids, denoted herein as PFeIFNα_{189b}, the amino acid sequence of which is presented in SEQ ID NO:111, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 and a last codon prior to a stop codon spanning from nucleotide 565 through nucleotide 567 of SEQ ID NO:110.

Translation of SEQ ID NO:155, the coding strand for nFeIFNα_{567c}, yields a protein of about 189 amino acids, denoted herein as PFeIFNα_{189c}, the amino acid sequence of which is presented in SEQ ID NO:156, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 and a last codon prior to a stop codon spanning from nucleotide 565 through nucleotide 567 of SEQ ID NO:155.

Translation of SEQ ID NO:161, the coding strand for nFeIFNα_{582d}, yields a protein of about 194 amino acids, denoted herein as PFeIFNα_{194d}, the amino acid sequence of which is presented in SEQ ID NO:162, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 and a last codon prior to a stop codon spanning from nucleotide 565 through nucleotide 567 of SEQ ID NO:161.

Translation of SEQ ID NO:167, the coding strand for nFeIFNα_{567e}, yields a protein of about 189 amino acids, denoted herein as PFeIFNα_{189e}, the amino acid sequence of which is presented in SEQ ID NO:168, assuming an open reading frame having an

initiation codon spanning from nucleotide 1 through nucleotide 3 and a last codon prior to a stop codon spanning from nucleotide 565 through nucleotide 567 of SEQ ID NO:167.

Translation of SEQ ID NO:119, the coding strand for $nFeGMCSF_{444}$, yields a protein of about 144 amino acids, denoted herein as $PFeGMCSF_{144}$, the amino acid sequence of which is presented in SEQ ID NO:120, assuming an open reading frame having an initiation codon spanning from nucleotide 10 through nucleotide 12 of SEQ ID NO:119 and a stop codon spanning from nucleotide 442 through nucleotide 444 of SEQ ID NO:119.

Preferred IL-4 proteins of the present invention include proteins that are at least about 85%, preferably at least about 90%, and even more preferably at least about 95% identical to $PCaIL\text{-}4_{132}$, $PCaIL\text{-}4_{108}$, or fragments thereof. Preferred Flt-3 ligand proteins of the present invention include proteins that are at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaFlt3L_{294}$, $PCaFlt3L_{268}$, $PCaFlt3L_{276}$, $PCaFlt3L_{250}$, $PCaFlt3L_{31}$, and/or fragments thereof. Additional preferred Flt-3 ligand proteins of the present invention includes proteins that are at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PFeFlt3L_{291}$, $PFeFlt3L_{265}$ and/or fragments thereof. Preferred CD40 proteins of the present invention includes proteins that are at least about 70%, preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaCD40_{274}$, $PCaCD40_{255}$ and/or fragments thereof. Additional

preferred CD40 proteins of the present invention includes proteins that are at least about 60%, at least about 65%, preferably at least about 70%, preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PFeCD40_{112}$ and/or fragments thereof. Preferred CD154 proteins of the present invention includes proteins that are at least about 80% identical, preferably at least about 85% identical, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaCD154_{260}$, $PCaCD154_{211}$ and/or fragments thereof. Additional preferred CD154 proteins of the present invention includes proteins that are at least about 85% identical, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PFeCD154_{260}$, $PFeCD154_{211}$ and/or fragments thereof. Preferred IL-5 proteins of the present invention includes proteins that are at least about 85% identical, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaIL\text{-}5_{134}$, $PCaIL\text{-}5_{115}$ and/or fragments thereof. Preferred IL-13 proteins of the present invention includes proteins that are at least about 70% identical, preferably at least about 75% identical, more preferably at least about 80% identical, more preferably at least about 85% identical, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaIL\text{-}13_{131}$, $PCaIL\text{-}13_{111}$, $PCaIL\text{-}13_{130}$, $PCaIL\text{-}13_{110}$, and/or fragments thereof. Preferred IFNα proteins of the present invention include $PFeIFN\alpha_{189a}$, $PFeIFN\alpha_{189b}$, $PFeIFN\alpha_{189c}$, $PFeIFN\alpha_{166a}$, $PFeIFN\alpha_{166c}$, $PFeIFN\alpha_{194d}$, $PFeIFN\alpha_{171d}$, $PFeIFN\alpha_{189e}$, $PFeIFN\alpha_{166e}$, and/or $PFeIFN\alpha_{166b}$. Preferred GM-CSF proteins of the present invention include $PFeGMCSF_{144}$, and/or $PFeGMCSF_{127}$.

More preferred are IL-4 proteins comprising PCaIL-4_{132}, PCaIL-4_{108}, and/or proteins encoded by allelic variants of a nucleic acid molecule encoding proteins PCaIL-4_{132} and/or PCaIL-4_{108}. More preferred are Flt-3 ligand proteins comprising PCaFlt3L_{294}, PCaFlt3L_{268}, PCaFlt3L_{276}, PCaFlt3L_{250}, PCaFlt3L_{31}, PFeFlt3L_{291}, PFeFlt3L_{265} and/or proteins encoded by allelic variants of a nucleic acid molecule encoding proteins PCaFlt3L_{294}, PCaFlt3L_{268}, PCaFlt3L_{276}, PCaFlt3L_{250}, PCaFlt3L_{31}, PFeFlt3L_{291}, and/or PFeFlt3L_{265}. More preferred are CD40 proteins comprising PCaCD40_{274}, PCaCD40_{255}, and/or PFeCD40_{112} and/or proteins encoded by allelic variants of a nucleic acid molecule encoding proteins PCaCD40_{274}, PCaCD40_{255}, and/or PFeCD40_{112}. More preferred are CD154 proteins comprising PCaCD154_{260}, PCaCD154_{211}, PFeCD154_{260}, PFeCD154_{211} and/or proteins encoded by allelic variants of a nucleic acid molecule encoding one of proteins PCaCD154_{260}, PCaCD154_{211}, PFeCD154_{260}, PFeCD154_{211}. More preferred are IL-5 proteins comprising PCaIL-5_{134}, PCaIL-5_{115} and/or proteins encoded by allelic variants of a nucleic acid molecule encoding one of the proteins PCaIL-5_{134} and/or PCaIL-5_{115}. More preferred are IL-13 proteins comprising PCaIL-13_{131}, PCaIL-13_{111}, PCaIL-13_{130}, PCaIL-13_{110}, and/or proteins encoded by allelic variants of anucleic acid molecule encoding one of the proteins PCaIL-13_{131}, PCaIL-13_{111}, PCaIL-13_{130}, PCaIL-13_{110}.

Also preferred are IL-4 proteins of the present invention having amino acid sequences that are at least about 85%, preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:2, SEQ ID NO:20 and/or fragments thereof. Also preferred are Flt-3 ligand proteins of the present invention having amino acid sequences that are at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even

more preferably at least about 95% identical to SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34 and/or fragments thereof. Additional preferred Flt-3 ligand proteins of the present invention includes proteins that are at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and/or even more preferably at least about 95% identical to SEQ ID NO:44, SEQ ID NO:49 and/or fragments thereof. Preferred CD40 proteins of the present invention includes proteins that are at least about 70%, preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and/or even more preferably at least about 95% identical to SEQ ID NO:53, SEQ ID NO:58 and/or fragments thereof. Additional preferred CD40 proteins of the present invention includes proteins that are at least about 60%, at least about 65%, preferably at least about 70%, preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:61 and/or fragments thereof. Preferred CD154 proteins of the present invention includes proteins that are at least about 80% identical, preferably at least about 85% identical, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:65, SEQ ID NO:70 and/or fragments thereof. Additional preferred CD154 proteins of the present invention includes proteins that are at least about 85% identical, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:73, SEQ ID NO:78 and/or fragments thereof. Preferred IL-5 proteins of the present invention includes proteins that are at least about 85% identical, even more preferably at

least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:81, SEQ ID NO:86 and/or fragments thereof. Preferred IL-13 proteins of the present invention includes proteins that are at least about 70% identical, preferably at least about 75% identical, more preferably at least about 80% identical, more preferably at least about 85% identical, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, SEQ ID NO:105, and/or fragments thereof. Preferred IFNα proteins of the present invention include SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:156, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:168, and SEQ ID NO:171. Preferred GM-CSF proteins of the present invention include SEQ ID NO:120, SEQ ID NO:125.

More preferred are IL-4 proteins comprising the amino acid sequence SEQ ID NO:2, SEQ ID NO:20; and/or IL-4 proteins encoded by allelic variants of nucleic acid molecules encoding IL-4 proteins having the amino acid sequence SEQ ID NO:2, SEQ ID NO:20. More preferred are Flt-3 ligand proteins comprising SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34, SEQ ID NO:44, SEQ ID NO:49 and/or proteins encoded by allelic variants of a nucleic acid molecule encoding proteins SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:44, and/or SEQ ID NO:49. More preferred are CD40 proteins comprising SEQ ID NO:53, SEQ ID NO:58, SEQ ID NO:61 and/or proteins encoded by allelic variants of a nucleic acid molecule encoding proteins SEQ ID NO:53, SEQ ID NO:58, and/or SEQ ID NO:61. More preferred are CD154 proteins comprising SEQ ID NO:65, SEQ ID NO:70, SEQ ID NO:73, SEQ ID NO:78 and/or proteins encoded by allelic

variants of a nucleic acid molecule encoding one of proteins SEQ ID NO:65, SEQ ID NO:70, SEQ ID NO:73, and/or SEQ ID NO:78. More preferred are IL-5 proteins comprising SEQ ID NO:81, SEQ ID NO:86 and/or proteins encoded by allelic variants of a nucleic acid molecule encoding one of the proteins SEQ ID NO:81, and/or SEQ ID NO:86. More preferred are IL-13 proteins comprising SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, SEQ ID NO:105, and/or proteins encoded by allelic variants of anucleic acid molecule encoding one of the proteins SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105.

Percent identities between amino acid or nucleic acid sequences can be determined using standard methods known to those of skill in the art. It is known in the art that methods to determine the percentage identity and the number of gaps are substantially similar when different methods for determining sequence similarity are used and when the degree of similarity is greater than 30% amino acid identity, as described by Johnson et al., *J. Mol. Biol.*, vol. 233, pages 716-738, 1993, and Feng et al., *J. Mol. Evol.*, vol. 21, pages 112-125, 1985, which are incorporated by reference herein in their entirety. Preferred methods to determine percentage identities between amino acid sequences and between nucleic acid sequences include comparisons using various computer programs such as GCG™ program (available from Genetics Computer Group, Madison, WI), DNAsis™ program (available from Hitachi Software, San Bruno, CA) or the MacVector™ program (available from the Eastman Kodak Company, New Haven, CT). Preferred settings for sequence comparisons using the DNAsis™ computer program or the GAP GCG™ program are disclosed herein in the Examples section.

Additional preferred IL-4 proteins of the present invention include proteins encoded by nucleic acid molecules comprising at least a portion of nCaIL-4_{549}, nCaIL-4_{396}, and/or nCaIL-4_{324}, as well as IL-4 proteins encoded by allelic variants of such nucleic acid molecules. Additional preferred Flt-3 ligand proteins of the present invention include proteins encoded by nucleic acid molecules comprising at least a portion of nCaFlt3L_{1013}, nCaFlt3L_{882}, nCaFlt3L_{804}, nCaFlt3L_{828}, nCaFlt3L_{985}, nCaFlt3L_{1019}, nCaFlt3L_{93}, nCaFlt3L_{750}, nFeFlt3L_{395}, nFeFlt3L_{793}, nFeFlt3L_{942}, nFeFlt3L_{873}, and/or nFeFlt3L_{795} as well as Flt-3 ligand proteins encoded by allelic variants of such nucleic acid molecules. Additional preferred CD40 proteins of the present invention include proteins encoded by nucleic acid molecules encoding at least a portion of nCaCD40_{321}, nCaCD40_{1425}, nCaCD40_{822}, nCaCD40_{765}, and/or nFeCD40_{336} as well as CD40 proteins encoded by allelic variants of such nucleic acid molecules. Additional preferred CD154 proteins of the present invention include proteins encoded by nucleic acid molecules encoding at least a portion of nCaCD154_{390}, nCaCD1541_{878}, nCaCD154_{780}, nCaCD154_{633}, nFeCD154_{885}, nFeCD154_{780}, and/or nFeCD154_{633} as well as CD154 proteins encoded by allelic variants of such nucleic acid molecules. Additional preferred IL-5 proteins of the present invention include proteins encoded by nucleic acid molecules encoding at least a portion of nCaIL-5_{610}, nCaIL-5_{402}, and/or nCaIL-5_{345} as well as IL-5 proteins encoded by allelic variants of such nucleic acid molecules. Additional preferred IL-13 proteins of the present invention include proteins encoded by nucleic acid molecules encoding at least a portion of nCaIL-5_{610}, nCaIL-5_{402}, and/or nCaIL-5_{345} as well as IL-13 proteins encoded by allelic variants of such nucleic acid molecules.

Also preferred are IL-4 proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:1, SEQ ID NO:4, and/or SEQ ID NO:19, as well as allelic variants of these nucleic acid molecules. Also preferred are Flt-3 ligand proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:46, and/or SEQ ID NO:48, as well as allelic variants of these nucleic acid molecules. Also preferred are CD40 proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:57, and/or SEQ ID NO:60, as well as allelic variants of these nucleic acid molecules. Also preferred are CD154 proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:75, and/or SEQ ID NO:77, as well as allelic variants of these nucleic acid molecules. Also preferred are IL-5 proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:80, SEQ ID NO:83, and/or SEQ ID NO:85, as well as allelic variants of these nucleic acid molecules. Also preferred are IL-13 proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:99, SEQ ID NO:102, and/or SEQ ID NO:104, as well as allelic variants of these nucleic acid molecules.

Another embodiment of the present invention is a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecule that includes one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the complementary sequence of another nucleic acid molecule. As such, the minimal size of a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecule of the present invention is from about 12 to about 18 nucleotides in length.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, and/or feline GM-CSF, nucleic acid molecules can include, for example,

natural allelic variants and/or nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, and/or feline GM-CSF protein of the present invention.

A canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, and/or feline GM-CSF ligand nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with either a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecule or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40,

canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein, respectively).

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF ligand protein.

A preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of regulating an immune response in an animal. As will be disclosed in more detail below, such a nucleic acid molecule can be, or encode, an antisense RNA, a molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode an immunoregulatory protein (e.g., a cell-bound or soluble protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e, as a genetic vaccine) or in a vehicle such as a recombinant virus vaccine or a recombinant cell vaccine.

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One embodiment of the present invention is an IL-4 nucleic acid molecule comprising all or part (i.e., a fragment of the IL-4 nucleic acid molecule) of nucleic acid molecules nCaIL-4_{549}, nCaIL-4_{396}, and/or nCaIL-4_{324}, or allelic variants of these nucleic acid molecules. One embodiment of the present invention is a Flt-3 ligand nucleic acid molecule comprising all or part (i.e., a fragment of the Flt-3 ligand nucleic acid molecule) of nucleic acid molecules nCaFlt3L_{1013}, nCaFlt3L_{882}, nCaFlt3L_{804}, nCaFlt3L_{828}, nCaFlt3L_{985}, nCaFlt3L_{1019}, nCaFlt3L_{93}, nCaFlt3L_{750}, nFeFlt3L_{395}, nFeFlt3L_{793}, nFeFlt3L_{942}, nFeFlt3L_{873}, and/or nFeFlt3L_{795} and/or allelic variants of these nucleic acid molecules. One embodiment of the present invention is a CD40 nucleic acid molecule comprising all or part (i.e. a fragment of the CD40 nucleic acid molecule) of nucleic acid molecules nCaCD40_{321}, nCaCD40_{1425}, nCaCD40_{822}, nCaCD40_{765}, and/or nFeCD40_{336} and/or allelic variants of these nucleic acid molecules. One embodiment of the present invention is a CD154 nucleic acid molecule comprising all or part of nucleic acid molecules nCaCD154_{390}, nCaCD1541_{878}, nCaCD154_{780}, nCaCD154_{633}, nFeCD154_{885}, nFeCD154_{780}, and/or nFeCD154_{633}, and/or allelic variants of these nucleic acid molecules. One embodiment of the present invention is an IL-5 nucleic acid molecule comprising all or part of nucleic acid molecules nCaIL-5_{610}, nCaIL-5_{402}, and/or nCaIL-5_{345}, and/or allelic variants of these nucleic acid molecules. One embodiment of the present invention is an IL-13 nucleic acid molecule comprising all or part of nucleic acid molecules nCaIL-13_{166}, nCaIL-13_{272}, nCaIL-13_{278}, nCaIL-13_{1302}, nCaIL-13_{393}, nCaIL-13_{333}, nCaIL-13_{1269}, nCaIL-13_{390}, and/or nCaIL-13_{330}, and/or allelic variants of these nucleic acid molecules. Another preferred nucleic acid molecule of the present invention includes at least a portion of (i.e., a fragment of the nucleic acid molecule) nucleic acid sequence SEQ ID NO:1, SEQ ID

NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:170, and/or SEQ ID NO:172, as well as allelic variants of nucleic acid molecules having these nucleic acid sequences. Such nucleic acid molecules can include nucleotides in addition to those included in the SEQ ID NOs, such as, but not limited to, a

full-length gene, a full-length coding region, a nucleic acid molecule encoding a fusion protein, and/or a nucleic acid molecule encoding a multivalent therapeutic compound.

One embodiment of an isolated nucleic acid molecule of the present invention is a nucleic acid molecule that can be any of the following: (a) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, and/or SEQ ID NO:21 and/or a homolog thereof, wherein said homolog has an at least 50 contiguous nucleotide region identical in sequence to a 50 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, and/or SEQ ID NO:21; (b) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, and/or SEQ ID NO:37, and/or a homolog thereof, wherein said homolog has an at least 40 contiguous nucleotide region identical in sequence to a 40 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, and/or SEQ ID NO:37; (c) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, and/or SEQ ID NO:50, and/or a

homolog thereof, wherein said homolog has an at least 30 contiguous nucleotide region identical in sequence to a 30 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, and/or SEQ ID NO:50; (d) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and/or SEQ ID NO:59, and/or a homolog thereof, wherein said homolog has an at least 40 contiguous nucleotide region identical in sequence to a 40 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and/or SEQ ID NO:59; (e) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:60 and/or SEQ ID NO:62, and/or a homolog thereof, wherein said homolog has an at least 30 contiguous nucleotide region identical in sequence to a 30 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:60 and/or SEQ ID NO:62; (f) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69 and/or SEQ ID NO:71, and/or a homolog thereof, wherein said homolog has an at least 45 contiguous nucleotide region identical in sequence to a 45 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ

ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69 and/or SEQ ID NO:71; (g) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and/or SEQ ID NO:79, and/or a homolog thereof, wherein said homolog has an at least 35 contiguous nucleotide region identical in sequence to a 35 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and/or SEQ ID NO:79; (h) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and/or SEQ ID NO:87, and/or a homolog thereof, wherein said homolog has an at least 45 contiguous nucleotide region identical in sequence to a 45 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and/or SEQ ID NO:87; (i) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and/or SEQ ID NO:106, and/or a homolog thereof, wherein said homolog has an at least 15 contiguous nucleotide region identical in sequence to a 15 contiguous nucleotide region of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:88, SEQ ID NO:89,

SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and/or SEQ ID NO:106; (j) an isolated nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:170 and/or SEQ ID NO:172; and/or (k) an isolated nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, and/or SEQ ID NO:126. The phrase, a homolog having an at least "x" contiguous nucleotide region identical in sequence to an "x" contiguous nucleotide region of a nucleic acid molecule selected from the group consisting of SEQ ID NO:"y", refers to an "x"-nucleotide in length nucleic acid molecule that is identical in sequence to an "x"-nucleotide portion of SEQ ID NO:"y", as well as to nucleic acid molecules that are longer in length than "x". The additional length may be in the form of nucleotides that extend from either the 5' or the 3' end(s) of the contiguous identical "x"-nucleotide portion. The 5' and/or 3' extensions can include one or more extensions that have no identity to an immunoregulatory molecule of the present invention, as well as extensions that show similarity or identity to cited nucleic acids sequences or portions thereof.

In another embodiment, an isolated nucleic acid molecule of the present invention can be any of the following: (a) a nucleic acid molecule having a nucleic acid sequence

encoding an IL-4 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20 and/or (ii) a protein comprising a fragment of at least 20 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20; (b) a nucleic acid molecule having a nucleic acid sequence encoding a Flt-3 ligand protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34, and/or (ii) a protein comprising a fragment of at least 25 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34; (c) a nucleic acid molecule having a nucleic acid sequence encoding a Flt-3 ligand protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 75 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:44 and/or SEQ ID NO:49 and/or (ii) a protein comprising a fragment of at least 25 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:44 and/or SEQ ID NO:49; (d) a nucleic acid molecule having a nucleic acid sequence encoding a CD40 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 70 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:53 and/or SEQ ID NO:58 and/or (ii) a protein comprising a fragment of at least 30 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:53 and/or SEQ ID NO:58; (e) a nucleic acid

molecule having a nucleic acid sequence encoding a CD40 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 60 percent identical to an amino acid sequence comprising SEQ ID NO:61 and/or (ii) a protein comprising a fragment of at least 20 amino acids of an amino acid sequence comprising SEQ ID NO:61; (f) a nucleic acid molecule having a nucleic acid sequence encoding a CD154 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 80 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:65 and/or SEQ ID NO:70, and/or (ii) a protein comprising a fragment of at least 35 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:65 and/or SEQ ID NO:70; (g) a nucleic acid molecule having a nucleic acid sequence encoding a CD154 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:73 and/or SEQ ID NO:78, and/or (ii) a protein comprising a fragment of at least 50 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:73 and/or SEQ ID NO:78; (h) a nucleic acid molecule having a nucleic acid sequence encoding an IL-5 protein selected from the group consisting of (i) a protein having an amino acid sequence that is at least about 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86 and/or (ii) a protein comprising a fragment of at least 20 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86; (i) a nucleic acid molecule having a nucleic acid sequence encoding an IL-13 protein selected from the group consisting of (i) a protein having an amino acid sequence that is

at least about 70 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105 and/or (ii) a protein comprising a fragment of at least 15 amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105; (j) a nucleic acid molecule having a nucleic acid sequence encoding an interferon alpha protein having an amino acid sequence that is selected from the group consisting of amino acid sequence SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:156, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:168, and/or SEQ ID NO:171; (k) a nucleic acid molecule having a nucleic acid sequence encoding a GMCSF protein having an amino acid sequence that is selected from the group consisting of amino acid sequence SEQ ID NO:120, SEQ ID NO:125, and/or (l) a nucleic acid molecule comprising a complement of any of the before-mentioned nucleic acid sequences; wherein said IL-4 protein elicits an immune response against an IL-4 protein selected from the group consisting of SEQ ID NO:2 and/or SEQ ID NO:20 and/or is a protein with interleukin-4 activity, said Flt-3 ligand protein elicits an immune response against a Flt-3 ligand protein selected from the group consisting of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:44, and/or SEQ ID NO:49 and/or is a protein with Flt-3 ligand activity, said CD40 protein elicits an immune response against a CD40 protein selected from the group consisting of SEQ ID NO:53, SEQ ID NO:58, and/or SEQ ID NO:61 and/or is a protein with CD40 activity, said CD154 protein elicits an immune response against a CD154 protein selected from the group consisting of SEQ ID NO:65, SEQ ID NO:70, SEQ ID NO:73, and/or SEQ ID NO:78 and/or is a protein with CD154 activity,

said IL-5 protein elicits an immune response against a IL-5 protein selected from the group consisting of SEQ ID NO:81 and/or SEQ ID NO:86 and/or is a protein with IL-5 activity, said IL-13 protein elicits an immune response against an IL-13 protein selected from the group consisting of SEQ ID NO:92, SEQ ID NO:97, SEQ ID NO:100, and/or SEQ ID NO:105 and/or is a protein with IL-13 activity, said interferon alpha protein elicits an immune response against an interferon alpha protein selected from the group consisting of SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:156, SEQ ID NO:159, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:168, and/or SEQ ID NO:171 and/or is a protein with interferon alpha activity, and said GMCSF protein elicits an immune response against a GMCSF protein selected from the group consisting of SEQ ID NO:120 and/or SEQ ID NO:125 and/or is a protein with GM-CSF activity.

In one embodiment, an IL-4 nucleic acid molecule of the present invention encodes a protein that is at least about 85%, preferably at least about 90%, preferably at least about 92%, and even more preferably at least about 95% identical to $PCaIL\text{-}4_{132}$ and/or $PCaIL\text{-}4_{108}$. In one embodiment, a Flt-3 ligand nucleic acid molecule of the present invention encodes a protein that is at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaFlt3L_{294}$, $PCaFlt3L_{268}$, $PCaFlt3L_{276}$, $PCaFlt3L_{250}$, and/or $PCaFlt3L_{31}$. In one embodiment, a Flt-3 ligand nucleic acid molecule of the present invention encodes a protein that is at least about 75%, more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95%

identical to $PFeFlt3L_{291}$, and/or $PFeFlt3L_{265}$. In one embodiment, a CD40 nucleic acid molecule of the present invention encodes a protein that is at least about $PCaCD40_{274}$, and/or $PCaCD40_{255}$. In one embodiment, a CD40 nucleic acid molecule of the present invention encodes a protein that is at least about 60%, preferably at least about 65%, preferably at least about 70%, preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to $PFeCD40_{112}$. In one embodiment, a CD154 nucleic acid molecule of the present invention encodes a protein that is at least about 80%, at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaCD154_{260}$, and/or $PCaCD154_{211}$. In one embodiment, a CD154 nucleic acid molecule of the present invention encodes a protein that is at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to $PFeCD154_{260}$, $PFeCD154_{211}$. In one embodiment, an IL-5 nucleic acid molecule of the present invention encodes a protein that is at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaIL\text{-}5_{134}$, and/or $PCaIL\text{-}5_{115}$. In one embodiment, an IL-13 nucleic acid molecule of the present invention encodes a protein that is at least about 70%, at least about 75%, at least about 80%, preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to $PCaIL\text{-}13_{131}$, $PCaIL\text{-}13_{111}$, $PCaIL\text{-}13_{130}$, $PCaIL\text{-}13_{110}$. Even more preferred is a nucleic acid molecule encoding $PCaIL\text{-}4_{132}$, $PCaIL\text{-}4_{108}$, $PCaFlt3L_{294}$, $PCaFlt3L_{268}$, $PCaFlt3L_{276}$, $PCaFlt3L_{250}$, $PCaFlt3L_{31}$, $PFeFlt3L_{291}$, $PFeFlt3L_{265}$, $PCaCD40_{274}$, $PCaCD40_{255}$, $PFeCD40_{112}$, $PCaCD154_{260}$, $PCaCD154_{211}$, $PFeCD154_{260}$, $PFeCD154_{211}$, $PCaIL\text{-}5_{134}$,

PCaIL-5_{115}, PCaIL-13_{131}, PCaIL-13_{111}, PCaIL-13_{130}, PCaIL-13_{110} and/or an allelic variant of such a nucleic acid molecule.

In another embodiment, an IL-4 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 85%, preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:2, SEQ ID NO:20. The present invention also includes an IL-4 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:2, and/or SEQ ID NO:20, as well as allelic variants of an IL-4 nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a Flt-3 ligand nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34. The present invention also includes a Flt-3 ligand nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:7, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:31, and/or SEQ ID NO:34, as well as allelic variants of a Flt-3 ligand nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a Flt-3 ligand nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 75%, more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:44, and/or SEQ ID NO:49. The present invention also includes a Flt-3 ligand nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:44, and/or SEQ ID NO:49, as well as allelic variants of a Flt-3 ligand nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a CD40 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 70%, preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:53 and/or SEQ ID NO:58. The present invention also includes a CD40 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:53 and/or SEQ ID NO:58, as well as allelic variants of a CD40 nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a CD40 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 60%, preferably at least about 65%, preferably at least about 70%, preferably at least about 75%, even more

preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:60. The present invention also includes a CD40 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:60, as well as allelic variants of a CD40 nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a CD154 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about at least about 80%, at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:67, and/or SEQ ID NO:69. The present invention also includes a CD154 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:67, and/or SEQ ID NO:69, as well as allelic variants of a CD154 nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a CD154 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:72, SEQ ID NO:75, and/or SEQ ID NO:77. The present invention also includes a CD154 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:72, SEQ ID NO:75, and/or SEQ ID NO:77, as well as allelic variants of a

CD154 nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, an IL-5 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about at least about 85%, at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:80, SEQ ID NO:83, and/or SEQ ID NO:85. The present invention also includes an IL-5 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:80, SEQ ID NO:83, and/or SEQ ID NO:85, as well as allelic variants of an IL-5 nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, an IL-13 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about at least about 70%, at least about 75%, at least about 80%, preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:99, SEQ ID NO:102, and/or SEQ ID NO:104. The present invention also includes an IL-13 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:99, SEQ ID NO:102, and/or SEQ ID NO:104, as well as allelic variants of an IL-13 nucleic acid molecule encoding a protein having these

sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, an IL-4 nucleic acid molecule of the present invention is at least about 90%, and preferably at least about 95% identical to nCaIL-4_{549}. Even more preferred is a nucleic acid molecule comprising nCaIL-4_{549}, nCaIL-4_{396}, nCaIL-4_{324}, and/or an allelic variant of such a nucleic acid molecule. In another embodiment, a Flt-3 ligand nucleic acid molecule of the present invention is at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to nCaFlt3L_{1013}. Even more preferred is a nucleic acid molecule comprising nCaFlt3L_{1013}, nCaFlt3L_{882}, nCaFlt3L_{804}, nCaFlt3L_{828}, nCaFlt3L_{985}, nCaFlt3L_{1019}, nCaFlt3L_{93}, and/or nCaFlt3L_{750}, and/or an allelic variant of such a nucleic acid molecule. In one embodiment, a Flt-3 ligand nucleic acid molecule of the present invention is at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to nFeFlt3L_{942}. Even more preferred is a nucleic acid molecule comprising nFeFlt3L_{395}, nFeFlt3L_{793}, nFeFlt3L_{942}, nFeFlt3L_{873}, and/or nFeFlt3L_{795}, and/or an allelic variant of such a nucleic acid molecule. In one embodiment, a CD40 nucleic acid molecule of the present invention is at least about 70%, at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to nCaCD40_{321}, nCaCD40_{1425}, nCaCD40_{822}, and/or nCaCD40_{765}, and/or an allelic variant of such a nucleic acid molecule. In one embodiment, a CD40 nucleic acid

molecule of the present invention is at least about 70%, at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to $nFeCD40_{336}$, and/or an allelic variant of such a nucleic acid molecule. In one embodiment, a CD154 nucleic acid molecule of the present invention is at least about 85%, preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to $nCaCD154_{390}$, $nCaCD1541_{878}$, $nCaCD154_{780}$, and/or $nCaCD154_{633}$, and/or an allelic variant of such a nucleic acid molecule. In one embodiment, a CD154 nucleic acid molecule of the present invention is at least about 91%, and preferably about 95% identical to $nFeCD154_{885}$, $nFeCD154_{780}$, and/or $nFeCD154_{633}$, and/or an allelic variant of such a nucleic acid molecule. In one embodiment, an IL-5 molecule of the present invention is at least about 90% and preferably at least about 95% identical to $nCaIL\text{-}5_{610}$, $nCaIL\text{-}5_{402}$, and/or $nCaIL\text{-}5_{345}$, and/or an allelic variant of such a nucleic acid molecule. In another embodiment, an IL-13 molecule of the present invention is at least about 65%, at least about 70%, preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to $nCaIL\text{-}13_{166}$, $nCaIL\text{-}13_{272}$, $nCaIL\text{-}13_{278}$, $nCaIL\text{-}13_{1302}$, $nCaIL\text{-}13_{393}$, $nCaIL\text{-}13_{333}$, $nCaIL\text{-}13_{1269}$, $nCaIL\text{-}13_{390}$, and/or $nCaIL\text{-}13_{330}$, and/or an allelic variant of such a nucleic acid molecule.

In another embodiment, an IL-4 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 90%, and preferably at least about 95% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, and/or SEQ ID NO:21. The present invention also includes an IL-4 nucleic acid

molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:19, and/or SEQ ID NO:21, as well as allelic variants of such IL-4 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a Flt-3 ligand nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, and/or SEQ ID NO:37. The present invention also includes a Flt-3 ligand- nucleic acid molecule comprising at least a portion of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, and/or SEQ ID NO:37, as well as allelic variants of such Flt-3 ligand nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a Flt-3 ligand nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to SEQ ID NO:41, SEQ ID NO:42,

SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, and/or SEQ ID NO:50. The present invention also includes a Flt-3 ligand- nucleic acid molecule comprising at least a portion of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, and/or SEQ ID NO:50, as well as allelic variants of such Flt-3 ligand nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a CD40 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 70%, at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and/or SEQ ID NO:59. The present invention also includes a CD40 nucleic acid molecule comprising at least a portion of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and/or SEQ ID NO:59, as well as allelic variants of such CD40 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a CD40 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 70%, at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to SEQ ID NO:60 and/or SEQ ID NO:62. The present invention also includes a CD40 nucleic acid

molecule comprising at least a portion of SEQ ID NO:60 and/or SEQ ID NO:62, as well as allelic variants of such CD40 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a CD154 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 85%, preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and/or SEQ ID NO:71. The present invention also includes a CD154 nucleic acid molecule comprising at least a portion of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and/or SEQ ID NO:71, as well as allelic variants of such CD154 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a CD154 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 91%, and preferably about 95% identical to SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and/or SEQ ID NO:79. The present invention also includes a CD154 nucleic acid molecule comprising at least a portion of SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and/or SEQ ID NO:79, as well as allelic variants of such CD154 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, an IL-5 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 90% and preferably at least about 95% identical to SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and/or SEQ ID NO:87. The present invention also includes an IL-5 nucleic acid molecule comprising at least a portion of SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and/or SEQ ID NO:87, as well as allelic variants of such IL-5 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, an IL-13 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 65%, at least about 70%, preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% and even more preferably at least about 95% identical to SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and/or SEQ ID NO:106. The present invention also includes an IL-13 nucleic acid molecule comprising at least a portion of SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, and/or SEQ ID NO:106, as well as allelic variants of such IL-13 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate

codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, an IFNα nucleic acid molecule of the present invention is identical to SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:170, and/or SEQ ID NO:172.

In another embodiment, a GM-CSF nucleic acid molecule of the present invention is identical to SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, and/or SEQ ID NO:126.

Knowing the nucleic acid sequences of certain immunoregulatory nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and/or (c) obtain other immunoregulatory nucleic acid molecules. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include mammalian cDNA libraries as well as

genomic DNA libraries. Similarly, preferred DNA sources from which to amplify nucleic acid molecules include mammalian cDNA and genomic DNA. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid.*

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. A preferred oligonucleotide of the present invention has a maximum size of about 100 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules, or therapeutic reagents to inhibit canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing therapeutic compositions can be administered to an animal using techniques known to those skilled in the art.

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating immunoregulatory nucleic acid molecules of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, parasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene

expression in bacterial, yeast, insect and mammalian cells, and more preferably in the cell types disclosed herein, more preferably *in vivo*.

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, helminth and/or other endoparasite, insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (such as lambda p_L and lambda p_R and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoter), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control

sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with mammals, such as dog, cat, horse or human transcription control sequences.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in recombinant vectors, and particularly in recombinant molecules, include nCaIL-4_{549}, nCaIL-4_{396}, nCaIL-4_{324}, nCaFlt3L$_{1013}$, nCaFlt3L$_{882}$, nCaFlt3L$_{804}$, nCaFlt3L$_{828}$, nCaFlt3L$_{985}$, nCaFlt3L$_{1019}$, nCaFlt3L$_{93}$, nCaFlt3L$_{750}$, nFeFlt3L$_{395}$, nFeFlt3L$_{793}$, nFeFlt3L$_{942}$, nFeFlt3L$_{873}$, nFeFlt3L$_{795}$, nCaCD40_{321}, nCaCD40_{1425}, nCaCD40_{822}, nCaCD40_{765}, nFeCD40_{336}, nCaCD154_{390}, nCaCD1541_{878}, nCaCD154_{780}, nCaCD154_{633}, nFeCD154_{885}, nFeCD154_{780}, nFeCD154_{633}, nCaIL-5_{610}, nCaIL-5_{402}, nCaIL-5_{345}, nCaIL-13_{166}, nCaIL-13_{272}, nCaIL-13_{278}, nCaIL-13_{1302}, nCaIL-13_{393}, nCaIL-13_{333}, nCaIL-13_{1269}, nCaIL-13_{390}, nCaIL-13_{330}, nFeIFNα_{567a}, nFeIFNα_{567b}, nFeIFNα_{567c}, nFeIFNα_{498a}, nFeIFNα_{498b}, nFeIFNα_{498c}, nFeIFNα_{582d}, nFeIFNα_{513d}, nFeIFNα_{567e}, nFeIFNα_{498e}, nFeGMCSF$_{444}$, nFeGMCSF$_{432}$, and/or nFeGMCSF$_{381}$.

Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed parasitic helminth protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid

molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteosome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include immunoregulatory nucleic acid molecules of the present invention disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include

nCaIL-4_{549}, nCaIL-4_{396}, nCaIL-4_{324}, nCaFlt3L_{1013}, nCaFlt3L_{882}, nCaFlt3L_{804}, nCaFlt3L_{828}, nCaFlt3L_{985}, nCaFlt3L_{1019}, nCaFlt3L_{93}, nCaFlt3L_{750}, nFeFlt3L_{395}, nFeFlt3L_{793}, nFeFlt3L_{942}, nFeFlt3L_{873}, nFeFlt3L_{795}, nCaCD40_{321}, nCaCD40_{1425}, nCaCD40_{822}, nCaCD40_{765}, nFeCD40_{336}, nCaCD154_{390}, nCaCD1541_{878}, nCaCD154_{780}, nCaCD154_{633}, nFeCD154_{885}, nFeCD154_{780}, nFeCD154_{633}, nCaIL-5_{610}, nCaIL-5_{402}, nCaIL-5_{345}, nCaIL-13_{166}, nCaIL-13_{272}, nCaIL-13_{278}, nCaIL-13_{1302}, nCaIL-13_{393}, nCaIL-13_{333}, nCaIL-13_{1269}, nCaIL-13_{390}, nCaIL-13_{330}, nFeIFNα_{567a}, nFeIFNα_{567b}, nFeIFNα_{567c}, nFeIFNα_{498a}, nFeIFNα_{498b}, nFeIFNα_{498c}, nFeIFNα_{582d}, nFeIFNα_{513d}, nFeIFNα_{567e}, nFeIFNα_{498e}, nFeGMCSF$_{444}$, nFeGMCSF$_{432}$, and/or nFeGMCSF$_{381}$.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing immunoregulatory proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including helminth, protozoa and ectoparasite), other insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, helminth, insect and mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Saccharomyces*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby

hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, chinese hamster ovary (CHO) cells, Ltk cells and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1 $_0$3987 and SR-11 $_0$4072; *Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK^{31} cells and/or HeLa cells. In one embodiment, the proteins may be expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein.

A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including any of canine interleukin-4, canine or feline Flt-3

ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF nucleic acid molecule encoding one or more proteins of the present invention and/or one or more other nucleic acid molecules encoding other therapeutic compounds, as disclosed herein (e.g., to produce multivalent vaccines).

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated immunoregulatory proteins of the present invention can be produced in a variety of ways, including production and/or recovery of natural proteins, production and/or recovery of recombinant proteins, and/or chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce an immunoregulatory protein of the present invention. Such medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional

steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and/or differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated animal.

The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to an immunoregulatory protein of the present invention and/ora mimetope thereof (e.g., anti-IL-4 antibodies, anti-Flt-3 ligand antibodies, anti-CD40 antibodies, anti-CD154 antibodies, anti-IL-5 antibodies, anti-IL-13 antibodies, anti-IFNα antibodies, and/or anti-GM-CSF antibodies). As used herein, the term "selectively binds to" an immunoregulatory protein of the present invention, refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and/or mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.*, is incorporated by this reference herein in its entirety. An anti-IL-4 antibody of

the present invention preferably selectively binds to an IL-4 protein in such a way as to inhibit the function of that protein. An anti-Flt-3 ligand antibody of the present invention preferably selectively binds to a Flt-3 ligand- protein in such a way as to inhibit the function of that protein. An anti-CD40 antibody of the present invention preferably selectively binds to a CD40 protein in such a way as to inhibit the function of that protein. An anti-CD154 antibody of the present invention preferably selectively binds to a CD154 protein in such a way as to inhibit the function of that protein. An anti-IL-5 antibody of the present invention preferably selectively binds to an IL-5 protein in such a way as to inhibit the function of that protein. An anti-IL-13 antibody of the present invention preferably selectively binds to an IL-13 protein in such a way as to inhibit the function of that protein. An anti-IFNα antibody of the present invention preferably selectively binds to an IFNα protein in such a way as to inhibit the function of that protein. An anti-GM-CSF antibody of the present invention preferably selectively binds to a GM-CSF protein in such a way as to inhibit the function of that protein.

Isolated antibodies of the present invention can include antibodies in serum, or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and/or genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide and/ormimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced

recombinantly using techniques as heretofore disclosed to produce any of the immunoregulatory proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as reagents in assays to detect an immunoregulatory protein of the present invention, (b) as reagents in assays to modulate cellular activity through an immunoregulatory protein of the present invention (e.g., mimicking ligand binding to a canine interleukin-4, canine or feline Flt-3 ligand, canine or feline CD40, canine or feline CD154, canine interleukin-5, canine interleukin-13, feline interferon alpha, or feline GM-CSF protein, as appropriate), and/or (c) as tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture of proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target compounds (e.g., nucleic acid molecules, drugs or proteins) to antigen presenting cells. Targeting can be accomplished by conjugating (i.e., stably joining) such antibodies to the compounds using techniques known to those skilled in the art. Suitable compounds are known to those skilled in the art.

One embodiment of the present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of regulating an immune response in an animal. Therapeutic compositions of the present invention can include at least one of the following therapeutic compounds: an isolated IL-4, Flt-3 ligand, CD40,

CD154, IL-5, IL-13, IFNα, and/or GM-CSF protein of the present invention and/or a mimetope thereof; an isolated IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF nucleic acid molecule of the present invention; an isolated antibody that selectively binds to an IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF protein of the present invention; an inhibitor of canine IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF function identified by its ability to bind to an IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF protein, respectively, of the present invention; such an inhibitor can inhibit binding of the respective immunoregulatory protein with its respective receptor, or inhibit the activity the respective protein. Methods to perform such assays to measure binding and/or activity of an immunoregulatory protein of the present invention are known to those of skill in the art, and are described, for example, in Janeway et al., *ibid.* As used herein, a therapeutic compound refers to a compound that, when administered to an animal in an effective manner, is able to treat, ameliorate, and/or prevent a disease. Examples of proteins, nucleic acid molecules, antibodies and/or inhibitors of the present invention are disclosed herein.

The present invention also includes a therapeutic composition comprising at least one IL-4-, Flt-3 ligand-, CD40-, CD154-, IL-5-, IL-13-, IFNα-, and/or GM-CSF-based compound of the present invention in combination with at least one additional therapeutic compound. Examples of such compounds are disclosed herein.

Therapeutic compositions of the present invention can be administered to any animal susceptible to such therapy, preferably to mammals, and more preferably to dogs,

cats, humans, ferrets, horses, cattle, sheep and/or other pets, economic food animals and/or zoo animals. Preferred animals include dogs, cats, horses and/or humans.

A therapeutic composition of the present invention is administered to an animal in an effective manner such that the composition is capable of regulating an immune response in that animal. Therapeutic compositions of the present invention can be administered to animals prior to onset of a disease (i.e., as a preventative vaccine) and/or can be administered to animals after onset of a disease in order to treat the disease (i.e., as a therapeutic vaccine). Preferred diseases to prevent and/or treat include autoimmune diseases, allergic reactions, infectious diseases, tumor development, inflammatory diseases and/or graft rejection. In one embodiment, a therapeutic composition of the present invnetion is administered with an antigen to enhance an immune response against that antigen.

Therapeutic compositions of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and/or other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and/or Tris buffer, while examples of preservatives include thimerosal, o-cresol, formalin and/or benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be

taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a therapeutic composition can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and/or compounds that induce the production of cytokines and/or chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

In one embodiment of the present invention, a therapeutic composition can include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain therapeutic dose levels of the composition to regulate an immune response in an animal. The therapeutic composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for

at least about 6 months, even more preferably for at least about 9 months, and even more preferably for at least about 12 months.

Therapeutic compositions of the present invention can be administered to animals prior to and/or after onset of disease. Acceptable protocols to administer therapeutic compositions in an effective manner include individual dose size, number of doses, frequency of dose administration, and/or mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of regulating the immune response in an animal when administered one or more times over a suitable time period. For example, a preferred single dose of a protein, mimetope or antibody therapeutic composition is from about 1 microgram (μg) to about 10 milligrams (mg) of the therapeutic composition per kilogram body weight of the animal. Booster vaccinations can be administered from about 2 weeks to several years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10 μg to about 1 mg of the therapeutic composition per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, subcutaneous, intradermal, intravenous, intranasal, intraoccular, oral, transdermal and/or intramuscular routes.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a therapeutic protein or therapeutic RNA (e.g., antisense RNA, ribozyme,

triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a naked (i.e., not packaged in a viral coat or cellular membrane) nucleic acid as a genetic vaccine (e.g., as naked DNA or RNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle).

A genetic (i.e., naked nucleic acid) vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention that preferably is replication, or otherwise amplification, competent. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a dicistronic recombinant molecule. Preferred genetic vaccines include at least a ortion of a viral genome (i.e., a viral vector). Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and/or retroviruses, with those based on alphaviruses (such as sindbis or Semliki forest virus), species-specific herpesviruses and/or poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of a "strong" polyadenylation signal is also preferred.

Genetic vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intranasal and/or oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 600 μg, depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, as drops, aerosolized and/or topically. Genetic vaccines of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) alone or in a carrier (e.g., lipid-based vehicles).

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and/or retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses (such as Sindbis virus), raccoon poxviruses, species-specific herpesviruses and/or species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus vaccines are disclosed in U.S. Patent Number 5,766,602 by Xiong et al., issued June 16, 1998, which is incorporated by this reference herein in its entirety.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a therapeutic protein or RNA nucleic acid molecule that is capable of protecting the animal from disease caused by a parasitic helminth as disclosed herein. For example, a

recombinant virus vaccine comprising an immunoregulatory nucleic acid molecule of the present invention is administered according to a protocol that results in the regulation of an immune response in an animal. A preferred single dose of a recombinant virus vaccine of the present invention is from about 1 x 10^4 to about 1 x 10^8 virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intranasal, intraoccular and/or oral administration routes being preferred.

A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast, (including *Saccharomyces cerevisiae and Pichia pastoris*), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK recombinant cells. Recombinant cell vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 10^8 to about 10^{12} cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

The efficacy of a therapeutic composition of the present invention to regulate the immune response in an animal can be tested in a variety of ways including, but not limited to, detection of cellular immunity within the treated animal, determining lymphocyte or dendritic cell activity, detection of immunoglobulin levels, determining hematopoietic stem cell or hematopoietic early progenitor cell development, determining

dendritic cell development or challenge of the treated animal with an infectious agent to determine whether the treated animal is resistant to disease. In one embodiment, therapeutic compositions can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

One embodiment of the present invention is an inhibitory compound. Preferably, such an inhibitory compound is derived from an IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF protein of the present invention. Examples of inhibitory compounds include an antibody of the present invention, that is administered to an animal in an effective manner (i.e., is administered in an amount so as to be present in the animal at a titer that is sufficient, upon interaction of that antibody with a native IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF protein, to decrease the activity of such proteins in an animal, at least temporarily). Oligonucleotide nucleic acid molecules of the present invention can also be administered in an effective manner, thereby reducing expression of either an IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF protein, in order to interfere with the protein activity targeted in accordance with the present invention. Peptides of an IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF protein of the present invention can also be administered in an effective manner, thereby reducing binding of IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF proteins to the appropriate receptor, in order to interfere with the protein activity targeted in accordance with the present invention. An inhibitory compound of an IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF function can be identified using IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF proteins of the present invention, respectively.

One embodiment of the present invention is a method to identify a compound capable of inhibiting IL-4 function. Such a method includes the steps of: (a) contacting (e.g., combining, mixing) an isolated IL-4 protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the IL-4 protein binds to IL-4 receptor or stimulates T cells in a T cell proliferation assay, and (b) determining if the putative inhibitory compound inhibits the binding of IL-4 protein to IL-4 receptor or the stimulation of T cells in a T cell proliferation assay. Another embodiment of the present invention is a method to identify a compound capable of inhibiting Flt-3 ligand function. Such a method includes the steps of: (a) contacting an isolated Flt-3 ligand protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the Flt-3 ligand protein binds to Flt-3 receptor or stimulates dendritic precursor cells in a proliferation assay, and (b) determining if the putative inhibitory compound inhibits the binding of Flt-3 ligand protein to Flt-3 receptor or the stimulation of dendritic precursor cells in a proliferation assay. Another embodiment of the present invention is a method to identify a compound capable of inhibiting CD40 function. Such a method includes the steps of (a) contacting an isolated CD40 protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the CD40 protein binds to a CD40 binding partner (e.g., CD154) and (b) determining if the putative inhibitory compound inhibits the binding of CD40 protein to the CD40 binding partner. A CD40 binding partner is a molecule that selectively binds to CD40 protein. Likewise, a binding partner for any other immunoregulatory protein of the present invention includes molecules that selectively bind to that particular immunoregulatory protein. Another

embodiment of the present invention is a method to identify a compound capable of inhibiting CD154 function. Such a method includes the steps of (a) contacting an isolated CD154 protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the CD154 protein binds to a CD154 binding partner (e.g., CD40) and (b) determining if the putative inhibitory compound inhibits the binding of CD154 protein to the CD154 binding partner. Yet another embodiment of the present invention is a method to identify a compound capable of inhibiting IL-5 function. Such a method includes the steps of: (a) contacting an isolated IL-5 protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the IL-5 protein binds to IL-5 receptor or stimulates T cells in a T cell proliferation assay, and (b) determining if the putative inhibitory compound inhibits the binding of IL-5 protein to IL-5 receptor or the stimulation of T cells in a T cell proliferation assay. Another embodiment of the present invention is a method to identify a compound capable of inhibiting IL-13 function. Such a method includes the steps of: (a) contacting an isolated IL-13 protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the IL-13 protein binds to IL-13 receptor or stimulates T cells in a T cell proliferation assay, and (b) determining if the putative inhibitory compound inhibits the binding of IL-13 protein to IL-13 receptor or the stimulation of T cells in a T cell proliferation assay. Another embodiment of the present invention is a method to identify a compound capable of inhibiting IFNα function. Such a method includes the steps of: (a) contacting an isolated IFNα protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the IFNα protein

binds to IFNα receptor or inhibits proliferation of GM-CSF stimulated TF-1 cells, and (b) determining if the putative inhibitory compound inhibits the binding of IFNα protein to IFNα receptor or inhibits proliferation of GM-CSF stimulated TF-1 cells. Another embodiment of the present invention is a method to identify a compound capable of inhibiting GM-CSF function. Such a method includes the steps of: (a) contacting an isolated GM-CSF protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of said compound, the GM-CSF protein binds to GM-CSF receptor or stimulates T cells in a T cell proliferation assay, and (b) determining if the putative inhibitory compound inhibits the binding of GM-CSF protein to GM-CSF receptor or the stimulation of T cells in a T cell proliferation assay.

Putative inhibitory compounds to screen include small organic molecules, antibodies (including mimetopes thereof), and/or ligand analogs. Such compounds are also screened to identify those that are substantially not toxic in host animals.

Preferred IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF, proteins to inhibit are those produced by dogs, cats, horses or humans, even more preferred IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF proteins to inhibit are those produced by domestic dogs or cats. A particularly preferred inhibitor of the present invention is capable of regulating an immune response in an animal. It is also within the scope of the present invention to use inhibitors of the present invention to target diseases involving undesired immune activity in animals. Compositions comprising inhibitors of IL-4, Flt-3 ligand, CD40, CD154, IL-5, IL-13, IFNα, and/or GM-CSF function can be administered to animals in an effective manner to regulate the immune response in the animals, and preferably to prevent autoimmune disease, allergy,

infectious disease, inflammation or prevent graft rejection in animals, or to treat animals with such diseases. Effective amounts and/or dosing regimens can be determined using techniques known to those skilled in the art.

It is also within the scope of the present invention to use isolated proteins, mimetopes, nucleic acid molecules and/or antibodies of the present invention as diagnostic reagents. Methods to use such diagnostic reagents are well known to those skilled in the art, see, for example, Janeway, et al., *ibid.*, and/or PCT Publication No. WO 98/23964, published June 4, 1998.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

It is to be noted that the examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be familiar to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.* and Ausubel, et al., 1993, *Current Protocols in Molecular Biology*, Greene/Wiley Interscience, New York, NY, and related references. Ausubel, et al, *ibid.* is incorporated by reference herein in its entirety.

Example 1

This example describes the isolation and sequencing of canine interleukin-4 (IL-4) nucleic acid molecules of the present invention. This example also describes expression of recombinant canine IL-4 in *E. coli* and mammalian cells; development of monoclonal and polyclonal antibodies to *E. coli* expressed canine IL-4; and bioactivity of mammalian-expressed and *E. coli*-expressed canine IL-4.

A. Isolation and sequencing of a canine IL-4 nucleic acid molecule.

Initial attempts to isolate a canine IL-4 nucleic acid molecule using various primers corresponding to putative conserved regions of IL-4 nucleic acid molecules failed. Forward and reverse primers were then designed using a sequence tag site (IL-4sts) described by Venta et al. in GenBank. The forward primer was designated as IL-4stsA, having the nucleic acid sequence 5' CTATTAATGG GTCTCACCTC CCAA CT 3', designated herein as SEQ ID NO:11. The reverse primer was designated as prIL-4stsB, having the nucleic acid sequence 5' TCAACTCGGT GCACAGAGTC TTGG 3', designated herein as SEQ ID NO:12. The primers were used to amplify PCR products from a *C. familiaris* mitogen activated PBMC cDNA library that was constructed in the Uni-ZAP® XR vector (available from Stratagene Cloning Systems, La Jolla, CA), using Stratagene's ZAP-cDNA® Synthesis Kit and the manufacturer's protocol. The mRNA was isolated from *C. familiaris* peripheral blood mononuclear cells about 4 hours after they were activated by a polyclonal activating agent in culture. Four PCR products were produced that had the expected size range. The PCR products were cloned and sequenced using standard techniques. A portion of one of the four products was found to be somewhat homologous with an IL-4 nucleic acid sequence reported in GenBank.

To identify a cDNA encoding a full-length canine IL-4 protein, the PCR product showing some homology with the IL-4 sequence reported in GenBank was used to generate an about 549 base pair DNA fragment as follows. The PCR product was labeled with ^{32}P and used as a probe to screen the canine PBMC cDNA library. Hybridization was done at about 6X SSC, 5X Denhardt's solution, 0.5 % SDS, 100 μg/ml of ssDNA and

100 μg/ml of tRNA, at about 68°C, for about 36 hr. (the compositions of SSC and Denhardt's are described in Sambrook et al., *ibid.*). The filters were washed 3 times, for about 30 minutes per wash, at about 55° C in about 2X SSC, 0.2% SDS, followed by a final wash of about 30 minutes in the same buffer except using about 1X SSC. Positive clones were isolated and the cDNA inserts were sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed using the GAP program of GCG (available from the University of Wisconsin) using the alignment settings of: gap weight set at 50, length weight set at 3, and average match set at 10 for nucleic acid sequence comparisons; and gap weight set at 12, length weight set at 4, and average match set at 2.912 for amino acid sequence comparisons.

A cDNA nucleic acid molecule was isolated, referred to herein as nCaIL-4_{549}, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:1. The complement of SEQ ID NO:1 is represented herein by SEQ ID NO:3. Translation of SEQ ID NO:1 suggests that nucleic acid molecule nCaIL-4_{549} encodes a full-length IL-4 protein of about 132 amino acids, denoted herein as PCaIL-4_{132}, the amino acid sequence of which is presented in SEQ ID NO:2, assuming an open reading frame having an initiation codon spanning from nucleotide 43 through nucleotide 45 of SEQ ID NO:1 and a stop codon spanning from nucleotide 439 through nucleotide 441 of SEQ ID NO:1. The coding region encoding PCaIL-4_{132} is presented herein as nCaIL-4_{396}, which has the nucleotide sequence SEQ ID NO:4 (the coding strand) and SEQ ID NO:5 (the complementary strand). A putative signal sequence coding region extends from nucleotide 43 through nucleotide 114 of SEQ ID NO:1. The proposed mature protein (i.e., canine IL-4 protein from which the signal sequence has been cleaved), denoted

herein as PCaIL-4_{108}, contains about 108 amino acids, extending from residue 25 through residue 132 of SEQ ID NO:2; PCaIL-4_{108} amino acid sequence is represented herein as SEQ ID NO:20. The nucleic acid molecule encoding PCaIL-4_{108} is denoted herein as nCaIL-4_{324}, extending from nucleotide 115 through nucleotide 438 of SEQ ID NO:1. nCaIL-4_{324} has a coding sequence denoted SEQ ID NO:19 and a complementary sequence denoted SEQ ID NO:21.

Comparison of nucleic acid sequence SEQ ID NO:1 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 89.3% identity, with a feline IL-4 gene. Comparison of amino acid sequence SEQ ID NO:2 with amino acid sequences reported in GenBank indicates that SEQ ID NO:2 showed the most homology, i.e., about 82.6% identity, with a feline IL-4 protein. Sequence analysis was performed using the GCG GAP program as described above.

B. Expression of recombinant canine IL-4 in *E. coli* and mammalian cells

i. *E. coli* expression

A recombinant molecule capable of expressing the mature form of canine IL-4, denoted herein as pGEX-nCaIL-4_{327}, was produced as follows. A 340-nucleotide fragment was PCR amplified from nucleic acid molecule nCaIL-4_{549} (having coding strand SEQ ID NO:1) using the following primer sequences: positive strand 5' T**GAATTC**GGA CATAACTTCA ATATTAC 3' (SEQ ID NO:38) (*Eco*RI site in bold) and 5' T**CTCGAG**ATT CAGCTTCATG CCTGTA 3' (SEQ ID NO39) (*Xho*I site in bold). The resulting 340-base pair fragment was digested with *Eco*RI and *Xho*I restriction enzymes (available from New England Biolabs, Beverly, MA), according to the

manufacturer's directions, and gel-purified using standard techniques. The digested 340-base pair fragment, now 327 base pairs, was then ligated into pGEX-6P-1 (available from Amersham Pharmacia, Piscataway, NJ), which had been previously digested with *Eco*RI and *Xho*I and gel purified, to produce recombinant molecule pGEX-nCaIL-4_{327}.

Recombinant molecules of pGEX produce the protein of interest as a glutathione s-transferase (GST) fusion protein. The recombinant molecule pGEX-nCaIL-4_{327} was transformed into DH5alpha cells (available from Life Technologies, Gaithersburg, MD), a recombination deficient strain of *E. coli*, to produce recombinant cell DH5-pGEX-nCaIL-4_{327}. The recombinant cells were screened for presence of insert by PCR and confirmed by enzyme restriction analysis and nucleic acid sequencing, using standard techniques. Several clonal recombinant molecules were transformed into BL21 cells (available from Amersham Pharmacia, Piscataway, NJ), a protease deficient strain of *E. coli*, to produce recombinant cell BL21-pGEX-nCaIL-4_{327}. These recombinant cells were screened, and the clone with the highest level of protein yield was selected for scaling up for larger-scale protein production. The resultant recombinant protein is referred to herein as *E. coli*PCaIL-4_{109}.

To produce and purify *E. coli*PCaIL-4_{109}, bacterial cultures of recombinant cell BL21:pGEX-nCaIL-4_{327} were grown in shake flasks at 37°C and induced with 0.1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), (available from Sigma Chemical Company, St. Louis, MO) when OD_{600nm} reached about 0.8 units. Growth was allowed to continue for about 4 hours; then bacteria were harvested by centrifugation at 4000 x g (times gravity) for 20 minutes. The bacterial pellet was washed and resuspended in phosphate buffered saline (PBS) (for recipe, see Sambrook et al, *ibid.*), then lysed by exposure to

gaseous nitrogen pressure in a Parr pressure vessel (available from Parr Instrument Co., Moline, IL), according to the manufacturer's instructions. Cell debris was removed by centrifugation at 10,000 x g for 20 minutes. The IL-4-GST fusion protein *E. coli*PCaIL-4_{109} was purified from the supernatant by allowing incubation with glutathione-conjugated resin, removing unbound proteins and then removing the GST tag with PRESCISSION™ protease; all reagents were available from Amersham Pharmacia and all were used according to the manufacturer's directions.

Concentration and purity of *E. coli*PCaIL-4_{109} were estimated by BCA Protein Assay kit (available from Pierce, Rockford, IL) and SDS-PAGE followed by Coomassie staining, respectively. The purified material exhibited a single band of approximately 14 kilodaltons (kD) by Coomassie stained SDS-PAGE.

ii. CHO cell expression

A recombinant molecule denoted herein as pCMV-nCaIL-4_{399}, capable of expressing a full length form of canine IL-4 (including signal sequence) was produced as follows. A 422-nucleotide fragment was PCR amplified from nucleic acid molecule nCaIL-4_{549} using the following primers: 5' CCC**AAGCTT**A TGGGTCTCACC TCCCAAC (*Hin*dIII site in bold), denoted SEQ ID NO:40, and 3' C**CTCGAG**ATT CAGCTTTCAA TGCCTGTA (*Xho*I site in bold), denoted SEQ ID NO:127. The 422-base pair PCR product was digested with the restriction endonucleases *Hin*dIII and *Xho*I, both available from New England Biolabs. The resulting 399-base pair product encoding full-length canine IL-4 was gel purified using standard techniques and ligated into the cytomegalovirus (CMV) immediate-early transcription control region of the pCMV-Int A

plasmid vector that had been digested with *Hin*dIII and *Xho*I (available from New England Biolabs), and gel purified, to produce the recombinant molecule pCMV-nCaIL-4_{399}. The pCMV-Int A plasmid vector was generated as referenced by J.E. Osorio et al., 1999, *Vaccine 17*, 1109-1116. Briefly, vector pRc/RSV, (available from Invitrogen Corp., San Diego, CA) was cleaved with restriction enzyme *Pvu*II (available from New England Biolabs), and the 2963-base pair *Pvu*II fragment was gel purified. The fragment was self-ligated to form the vector pRc/RSV(*Pvu*), which contains a Rous Sarcoma Virus (RSV) long terminal repeat, a multiple cloning site, a bovine growth hormone polyadenylation sequence, a bacterial origin of replication, and an ampicillin resistance gene. Vector pRc/RSV(*Pvu*) was restriction enzyme digested using *Hin*dIII and *Nru*I. A *Hin*dIII/*Ssp*I fragment containing the HCMV intermediate early promoter and first intron (i.e. intron A) was ligated into the digested pRc/RSV(*Pvu*) vector to produce the vector pCMV-Int A.

Stable expression of CaIL-4 in mammalian cells was carried out by transfecting the recombinant molecule pCMV-nCaIL-4_{399} into Chinese Hamster Ovary cells, (CHO, available from ATCC) as follows. Six-well polystyrene tissue culture plates (available from Corning Costar, Acton, MA) were seeded with approximately 5 x 10^5 cells/well in 2 milliliter (ml) cell culture media, consisting of Minimal Essential Media (MEM) supplemented with 100 mM L-glutamine, 100 mM gentamicin, and 10% fetal bovine serum (FBS), (all available from Life Technologies). Cells were grown to about 80% confluence (for about 18 hours) before transfection. The recombinant molecules to be transfected were purified using the Plasmid Midi Kit (available from Qiagen, Valencia, CA) and used according to the manufacturer's instructions. The recombinant molecule

pCMV-nCaIL-4_{399} was linearized using the restriction enzyme *Pvu*I (available from New England Biolabs). The plasmid pcDNA3, (available from Invitrogen), which contains the neomycin resistance gene, was linearized using the restriction enzyme *Eco*RI. Approximately 2 μg of pCMV-nCaIL-4_{399} was mixed with about 2 ng of linearized pcDNA3 in about 100 μl OPTIMEM™ media, available from Life Technologies. About 10 μl Lipofectamine, (available from Life Technologies) was mixed with 100 μl OPTIMEM. The nucleic acid molecule-containing mixture was then added to the Lipofectamine mixture and incubated at room temperature for about 45 minutes. After incubation, about 0.8 ml OPTIMEM was added, and the mixture was overlaid onto the CHO cells which had been previously rinsed with OPTIMEM. Cells were incubated for about 5 hours at 37°C 5% CO_2, 95% relative humidity. Approximately 1 ml of cell culture media as described previously, with 20% FBS, was added and the cells were incubated overnight. The media was changed at 24 hours, and at 72 hours post transfection, the cells were split 1:4 and put into fresh cell culture media containing about 500 μg/ml geneticin (G418, available from Life Technologies). The media was changed every 3-5 days. After several weeks, G418 resistant colonies were trypsinized using sterile filter papers, 5-6 mm in diameter that were soaked in trypsin, which were then placed over individual wells of 24 well plates that contained separated widely spaced colonies of CHO cells. After 3 days, the papers were removed. The resulting recombinant cells are referred to herein as CHO-pCMV-nCaIL-4_{399}. The recombinant cells were then expanded and tested for the presence of nIL-4_{399} RNA by RT-PCR and tested for the presence of PCaIL-4_{133} protein by Western blot analysis. Westerns were developed with rabbit anti-*E. coli*PCaIL-4_{109} serum and 607.1 monoclonal antibody, a

monoclonal antibody that selectively binds to *E. coli*PCaIL-4_{109} protein. See Example 1C for a description of how these antibodies were produced.

C. Monoclonal and polyclonal antibodies to recombinant canine IL-4 (i.e., anti-canine IL-4 antibodies)

The following describes the development of monoclonal and polyclonal antibodies that selectively bind to *E. coli*PCaIL-4_{109}.

Female Balb/C mice, 6-8 weeks old, were injected subcutaneously, at about 4 sites, with a total of 25μg *E. coli*PCaIL-4_{109} (produced as described in Example 1Bi) in Freund's Complete Adjuvant (day 0). Fourteen days later, the mice received an intraperitoneal boost of 25μg *E. coli*PCaIL-4_{109} in Freund's Incomplete Adjuvant (day 14). Fourteen days later, serum was tested for antibody titer to *E. coli*PCaIL-4_{109} by ELISA (day 28). Three days prior to fusion, mice were boosted intravenously with 20μg *E. coli*PCaIL-4_{109} in PBS (day 35). Splenocytes were harvested from mice demonstrating the highest serum titer by ELISA and depleted of CD4+ and CD8+ cells. This depletion was achieved by incubation of the splenocytes with biotinylated rat anti-mouse CD4 and anti-mouse CD8 monoclonal antibodies, available from PharMingen, San Diego, CA. Antibody-labeled cells were then removed by incubation with M-280 streptavidin coated magnetic beads, available from Dynal, Oslo, Norway. Depleted splenocytes were fused to SP2/0 cells (available from ATCC) using 50% polyethylene glycol in unsupplemented Iscove's Modified Dulbecco's Media (IMDM), following established protocols; see, for example, Harlow E., and Lane D., eds., 1995, *Antibodies. A Laboratory Manual*, Monoclonal Antibodies, Cold Spring Harbor Laboratories; Harlow et al, *ibid.*, is incorporated by reference herein in its entirety. Fused cells were plated in 96-well plates

using IMDM cell culture media, (available from Life Technologies, Inc., Rockville, MD), which was supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1 X nonessential amino acids, 1 X MEM amino acids, 0.05 mg/ml gentamicin, and 0.5 mM β-mercaptoethanol (all reagents available from Life Technologies). Additionally, 100 μM hypoxanthine, 0.4 μM aminopterin, and 16μM thymidine, all available from Sigma Chemical Corporation, St Louis, MO, were added.

After about 7 days, wells positive for hybridoma growth were screened by ELISA to *E. coli*PCaIL-4_{109}. Immulon II 96-well plates (available from VWR, Denver, CO) were coated, overnight, with 100 ng/ml *E. coli*PCaIL-4_{109} in 0.1 M carbonate/bicarbonate buffer, Ph 9.6. After blocking the wells with 20% FBS in Tris buffered saline (TBS), culture supernatants were allowed to bind. Presence of anti-*E. coli*PCaIL-4_{109} mouse antibody was detected with polyclonal goat anti-mouse IgG conjugated to horseradish peroxidase, (available from KPL, Gaithersburg, MD), and color developed with 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB), available from Pierce, Rockford, IL. Specificity of the ELISA reactivity was verified by Western blot analysis to *E. coli*PCaIL-4_{109}, developed with polyclonal goat anti-mouse IgG conjugated to alkaline phosphatase and nitro-blue tetrazolium/5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt substrate (NBT/BCIP, available from Sigma). Western blots exhibited a single band of approximately 14 kD. Immunoglobulin isotype of the monoclonal antibodies was determined using IsoStrips, available from Boehringer Mannheim, Indianapolis, IN. Twenty-three monoclonal antibodies were generated to *E. coli*PCaIL-4_{109}, 22 of which were of the IgM isotype and one of which was IgG1, and is referred to herein as 607.1.

Polyclonal rabbit serum was produced by repeated immunization (over a 10 month period) of a male, New Zealand White rabbit 12-16 months old. Initial immunization was 50 ug *E. coli*PCaIL-4_{109} (prepared as described in Example 1bi) in Freund's Complete Adjuvant, at several sites subcutaneously and intradermally. One month later, and at one month intervals thereafter, the rabbit was boosted intradermally with 50 ug *E. coli*PCaIL-4_{109} in Freund's Incomplete Adjuvant. Serum was collected bi-weekly and titers monitored by ELISA and Western blot to *E. coli*PCaIL-4_{109}. Serum that selectively bound to *E. coli*PCaIL-4_{109} protein is referred to as anti-*E. coli*PCaIL-4_{109} serum.

D. Bioactivity of mammalian-expressed canine IL-4

The following describes a bioassay to detect the expression of canine IL-4 protein expressed in the supernatants from CHO-pCMV-nCaIL-4_{399} recombinant cells by screening for production of CD23.

About 100 μl Ramos cells, available from ATCC, at a concentration of about 3.5 x 10^3 cells/ ml were seeded into 96-well flat bottom plates, available from Becton Dickinson, Franklin Lakes, NJ). These cells were grown in RPMI media supplemented with 100 mM L-glutamine, gentamicin, and 10 % FBS (called TCM). The Ramos cells were then treated in 5% CO_2 for 37°C for approximately 48 h. with one of the following:

Group	Treatment
1	TCM
2	CHO-pCMV (a transfectant cell line containing the empty pCMV vector) supernatant (1:4 final dilution in TCM)
3	CHO-pCMV-nCaIL-4_{399} supernatant (1:20 final dilution in TCM)

T1230

Triplicate samples for each treatment group were pooled for staining to look for increased expression of CD23 (one of the reported effects of IL-4). Briefly, 1 x 10^5 cells from each treatment group were incubated in phosphate buffered saline (PBS) containing 30% FBS for 15-30 min on ice. The cells were collected and incubated with the following:

Condition	Primary Incubation	Secondary Incubation
A	PBS	Goat anti mouse PE
B	Mouse anti human CD23	Goat anti mouse PE

Mouse anti-human CD23 monoclonal antibody, available from Pharmingen, was used at about 10 μg/ml. Goat (Fab'2) anti mouse IgG PE, available from Southern Biotechnologies was used at about 2.5 μg/ml. These reagents were diluted in PBS with 5% FBS . Primary incubations were performed for 30-60 minutes on ice, and secondary incubations were performed for 20-30 min on ice. Three washes of PBS/5% FBS were performed in between each incubation. Cells were then analyzed on a flow cytometer (e.g., MoFlow Desk Top System, available from Cytomation, Ft. Collins, CO) with the fluorescein gate set at 10^1. The results are shown in Table 2.

Table 2. Induction of CD23 on Ramos cells post-treatment with supernatants from CHO-pCMV-nCaIL-4_{399}.

Treatment	Condition	% positive
1	A B	0 1
2	A B	8 1
3	A B	3 99

Table 2 shows that the canine Il-4 expressed by the CHO transfectant CHO-pCMV-nCaIL-4_{399} is biologically active, demonstrated by its ability to induce expression of CD23 in Ramos cells.

E. Bioactivity of *E. coli*-expressed canine IL-4

The following describes a bioassay to detect the expression of canine IL-4 by stimulating the proliferation of TF-1 cells.

TF-1 cells (a human erythroleukaemia cell line, available from R&D Systems, Minneapolis, MN), were grown and maintained in TCM-TF-1 medium (RPMI-1640 media supplemented with 2 mM L-glutamine, 5 μg/ml gentamicin, 5% FBS and 2 ng/ml recombinant human GM-CSF (rhuGM-CSF, available from R&D Systems)) in 5% CO_2 at 37°C.

For assay, TF-1 cells were extensively washed to remove rhuGM-CSF, then added at approximately 1 x 10^4 cells per well to 96-well flat bottom plates. Refolded and HPLC-purified *E. coli*-expressed PCaIL-$4_{109,}$ produced as described in Example 1Bi, was diluted to the appropriate concentration in TCM-TF-1 without rhuGM-CSF and filter sterilized. Cells and *E. coli*-expressed PCaIL-4_{109} were incubated for 48 hours in 5% CO_2 at 37°C, then pulsed with 1 μCi/well tritiated thymidine (available from ICN Pharmaceuticals, Irvine, CA), and incubated for an additional 18 hours. Contents of the wells were harvested onto glass fiber filters and counted in a Wallac Trilux 1450 scintillation counter (available from Wallac Inc., Gaithersburg, MD). The results are shown in Table 3.

Table 3. Stimulation of proliferation of TF-1 cells with *E. coli*-expressed PCaIL-4_{109}

Concentration *E. coli* PcaIL-4_{109} (ng/ml)	Counts per minute
1000	33,216
500	26,297
250	27,283
125	23,804
62.5	26,225
31.3	19,803
15.6	9,818
7.8	6,475
0	165

Table 3 shows that canine IL-4 expressed by *E. coli* is biologically active, as demonstrated by its ability to stimulate proliferation of TF-1 cells.

Example 2

This example describes the isolation and sequencing of certain canine Flt-3 ligand and feline Flt-3 nucleic acid molecules and proteins of the present invention. The example also describes expression of a canine Flt-3 ligand protein of the present invention in CHO cells, as well as detection of the expressed canine Flt-3 ligand protein.

A. Canine Flt-3 ligand nucleic acid molecules and proteins.

i. This example describes the isolation and sequencing of certain canine Flt-3 ligand nucleic acid molecules and proteins of the present invention.

A canine Flt-3 ligand nucleic acid molecule was produced as follows. A pair of primers was initially used to amplify DNA from the *C. familiaris* mitogen activated PBMC cDNA library described above in Example 1. A forward primer referred to as FLT3F1, having the nucleic acid sequence 5' CTGGCGCCAG CCTGGAGCCC 3', designated herein as SEQ ID NO:13 was used in combination with a reverse primer

referred to herein as FLT3B1, having the nucleic acid sequence 5' GGGAGATGTT GGTCTGGACG 3', referred to herein as SEQ ID NO:14 to amplify Flt-3 ligand DNA from the cDNA library by polymerase chain reaction (PCR). The primers were designed using conserved regions of IL-4 cDNA sequences from other species in the public databases corresponding to the positions shown below:

Database	Accession number	Nucleotides	Animal
gb	U04806	102-121	human
gb	L23636	41-60	mouse
gb	U04806	77-458	human
gb	L23636	419-400	mouse

A 360-base pair (bp) PCR product was generated in the above reaction that was purified, radiolabeled and used as a probe to screen the cDNA library. Hybridization was performed in 6X SSC, 5X Denhardt's solution, 0.5 % SDS, 100 μg/ml ssDNA and 100 μg/ml of tRNA, at 68°C, for about 36 hr. The filters were washed 3 times, for about 30 minutes per wash, at 55°C in 2X SSC, 0.1% SDS, followed by a final wash in 0.25X SSC, for about 30 minutes, at 55° C. Several positive phage clones were identified and shown to produce PCR products when used as templates in combination with the FLT3F1 and FLT3B1 primers. The DNA inserts in the phage clones were sequenced using standard techniques and failed to yield any clones containing DNA inserts having a portion homologous to published Flt-3 ligand sequences. The 360-bp PCR fragment generated above was then cloned into the vector pcDNA 2.1 (available from Invitrogen Corp., San Diego, CA). Several independent colonies were generated and the sequences of their inserts determined. One clone was identified that which contained insert

sequence having a portion that was somewhat homologous to published human or murine Flt-3 ligand sequence.

Two canine Flt-3 ligand-specific primers were then designed using the nucleic acid sequence obtained using the 360-bp PCR product described above.

Primer	Sequence	SEQ ID NO
DFLB1	5' GACCAGGCGCCAGAACGC 3'	SEQ ID NO:15
DFLF1	5' CGGTCACCATCCGCAAGC 3'	SEQ ID NO:16

The 5' region of a Flt-3 ligand nucleic acid molecule was PCR amplified from the cDNA library using the DFLB1 primer in combination with the 5' T3 vector primer from the Uni-ZAP® XR vector (available from Stratagene). The 3' region of a Flt-3 ligand nucleic acid molecule was PCR amplified from the cDNA library using the DFLF1 in combination with the 3' T7 primer from the Uni-ZAP® XR vector (available from Stratagene). A 855-bp PCR product was obtained representing the 5' region of a Flt-3 ligand nucleic acid molecule. A 265-bp PCR product was obtained representing the 3' region of a Flt-3 ligand nucleic acid molecule. Both the 855-bp PCR product and 265-bp PCR product were cloned and sequenced using standard methods. Additional canine Flt-3 ligand-specific primers were designed using the nucleic acid sequence obtained from the sequence of the 855-bp PCR product and 265-bp PCR products.

Primer	Sequence	SEQ ID NO
DFLB2	5' TGGCAAGGCAGTGGCCTC 3'	SEQ ID NO:17
DFLF2	5' GCCGAGATGATAGTGCTGGC 3'	SEQ ID NO:18

A 546-bp PCR product was generated using the primer DFLF2 in combination with the primer DFLB2 to amplify a PCR product from the cDNA library. The 546-bp

PCR product was then purified, radiolabelled and used as a probe to screen the cDNA library. Hybridization was performed in 6X SSC, 5X Denhardt's solution, 0.5 % SDS, 100 μg/ml of ssDNA and 100 μg/ml of tRNA, at 68°C, for about 36 hr. The filters were washed in 1.25X SSC, for about 30 minutes, at 55°C. Four cDNA clones encoding full-length canine Flt-3 ligand were isolated. Nucleic acid sequence was obtained using standard techniques.

A Flt-3 ligand clone was isolated, referred to herein as $nCaFlt3L_{1013}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:6. The complement of SEQ ID NO:6 is represented herein by SEQ ID NO:8. Translation of SEQ ID NO:6 suggests that nucleic acid molecule $nCaFlt3L_{1013}$ encodes a full-length Flt-3 ligand protein of about 294 amino acids, denoted herein as $PCaFlt3L_{294}$, the amino acid sequence of which is presented in SEQ ID NO:7, assuming an open reading frame having an initiation codon spanning from nucleotide 35 through nucleotide 37 of SEQ ID NO:6 and a stop codon spanning from nucleotide 917 through nucleotide 919 of SEQ ID NO:6. The coding region encoding $PCaFlt3L_{294}$ is presented herein as $nCaFlt3L_{882}$, which has the nucleotide sequence SEQ ID NO:9 (the coding strand) and SEQ ID NO:10 (the complementary strand). A putative signal sequence coding region extends from nucleotide 35 through nucleotide 112 of SEQ ID NO:6. The proposed mature protein (i.e., canine Flt-3 ligand protein from which the signal sequence has been cleaved), denoted herein as $PCaFlt3L_{268}$ (SEQ ID NO:23), contains about 268 amino acids, extending from residue 27 through residue 294 of SEQ ID NO:7. The nucleic acid molecule encoding $PCaFlt3L_{268}$ is denoted herein as $nCaFlt3L_{804}$, extending from nucleotide 113 through nucleotide 916 of SEQ ID NO:6. $nCaFlt3L_{804}$ has a coding

sequence denoted SEQ ID NO:22 and a complementary sequence denoted SEQ ID NO:24.

Below is a description of the identification of alternatively spliced *Canis* Flt3 ligand transcripts. Besides cDNA clones such as nucleic acid molecule $nCaFlt3L_{1013}$ encoding the full-length canine Flt3 ligand protein, two splice variants of canine Flt3 ligand cDNA clones were also isolated, using the same hybridization conditions as mentioned previously in this Example. One such variant (Clone 1), denoted herein as $nCaFlt3L_{985}$, has a coding strand the nucleic acid sequence of which is represented as SEQ ID NO:25. The complement of SEQ ID NO:25 is represented herein by SEQ ID NO:27. Translation of SEQ ID NO:25 suggests that nucleic acid molecule $nCaFlt3L_{985}$ encodes a Flt-3 ligand protein of 276 amino acids, denoted herein as $PCaFlt3L_{276}$, the amino acid sequence of which is represented by SEQ ID NO:26, assuming an open reading frame having an initiation codon spanning from nucleotide 74 through nucleotide 76 of SEQ ID NO:25 and a stop codon spanning from nucleotide 902 through nucleotide 904 of SEQ ID NO:25. The coding region encoding $PCaFlt3L_{276}$ is represented herein as $nCaFlt3L_{828}$, which has the nucleotide sequence SEQ ID NO:28 (the coding strand) and SEQ ID NO:29 (the complementary strand). Alignment of nucleic acid molecules $nCaFlt3L_{882}$ and $nCaFlt3L_{828}$ indicates that the nucleic acid molecules are identical except for a deletion in $nCaFlt3L_{828}$, which spans from nucleotide 343 through nucleotide 396 of $nCaFlt3L_{882}$. Accordingly, $nCaFlt3L_{828}$ encodes 18 fewer amino acids than $nCaFlt3L_{882}$. The deletion in $PCaFlt3L_{276}$, which spans from residue 115 through residue 132 of $PCaFlt3L_{294}$, occurs between helix III and helix IV of the canine Flt3 ligand protein inferred from alignment with the human and mouse Flt3 ligand protein (Lyman et al.,

Cell, vol. 75, pp. 1157-1167, 1993; Hannum et al., *Nature*, vol. 368, pp. 643-648, 1994; Lyamn et al., *Blood,* vol. 83, pp. 2795-2801, 1994). In addition, the alignment shows that there are 39 more nucleotides in the 5' untranslated region of nucleic acid molecule nCaFlt3L$_{985}$ (nucleotides 1 to 39) than nucleic acid molecule nCaFlt3L$_{1013}$ and there are 2 more nucleotides in the 3' untranslated region of nucleic acid molecule nCaFlt3L$_{985}$ (nucleotides 922 to 923) than nucleic acid molecule nCaFlt3L$_{1013}$. The remaining sequences between nCaFlt3L$_{985}$ and nCaFlt3L$_{1013}$ are identical. A putative mature form of nCaFlt3L$_{985}$ (without the signal sequence) is predicted. The putative signal sequence coding region extends from nucleotide 74 to nucleotide 151 of SEQ ID NO:25. The proposed mature protein, denoted herein as PCaFlt3L$_{250}$, represented by SEQ ID NO:31, contains about 250 amino acids, extending from residue 27 through residue 276 of SEQ ID NO:26. The nucleic acid molecule encoding PCaFlt3L$_{250}$, extending from nucleotide 152 through nucleotide 901 of SEQ ID NO:6, denoted herein as nCaFlt3L$_{750}$, is represented by SEQ ID NO:30 (the coding strand) and SEQ ID NO:32 (the complement strand).

A second variant (Clone 19) is represented by nucleic acid molecule nCaFlt3L$_{1019}$, the coding strand of which is denoted herein as SEQ ID NO:33. The complement of SEQ ID NO:33 is denoted herein as SEQ ID NO:35. Translation of SEQ ID NO:33 suggests that nCaFlt3L$_{1019}$ encodes a Flt-3 ligand protein of 31 amino acids, PCaFlt3L$_{31}$, denoted SEQ ID NO:34, assuming an initiation codon spanning from nucleotide 74 through nucleotide 76 and a stop codon spanning nucleotide 167 through nucleotide 169 of SEQ ID NO:33. The coding region encoding PCaFlt3L$_{31}$ is represented herein as nCaFlt3L$_{93}$, which has the nucleotide sequence SEQ ID NO:36 (the coding strand) and SEQ ID

NO:37 (the complementary strand). Alignment of nucleic acid molecules $nCaFlt3L_{985}$ and $nCaFlt3L_{1019}$ indicates the presence of an insertion of 91 nucleotides in $nCaFl3L_{1019}$. The insertion spans nucleotide 107 through nucleotide 198 of $nCaFlt3L_{1019}$. A stop codon is found in this insertion in frame with the predicted initiation codon, which span nucleotide 74 through nucleotide 76 of SEQ ID NO:6. Since this insertion (with an in-frame stop codon) occurs in or close to the signal peptide, it is likely that nucleic acid molecule $nCaFlt3L_{1019}$ encodes a nonfunctional Flt-3 ligand protein.

Comparison of nucleic acid sequence SEQ ID NO:6 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:6 showed the most homology, i.e., about 69.8% identity, with a human Flt-3 ligand gene. Comparison of amino acid sequence SEQ ID NO:7 with amino acid sequences reported in GenBank indicates that SEQ ID NO:7 showed the most homology, i.e. about 71% identity, with a human Flt-3 ligand protein. Sequence analysis was performed with DNAsis™ using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; K-tuple set at 2; window size set at 5 and floating gap penalty set at 10.

ii. This example describes the production of a recombinant molecule encoding a full length canine Flt-3 ligand protein and expression of that protein by a recombinant cell of the present invention.

A recombinant molecule, denoted herein as pCMV-$nCaFlt3L_{882}$ and capable of expressing a full length form of Flt-3 ligand, was produced as follows. Nucleic acid molecule $nCaFlt3L_{882}$ was digested with the restriction endonucleases *Eco*RI and *Xba*I, gel purified and ligated into pCMV-Int A (prepared by methods described in Example 1)

to produce recombinant molecule pCMV-nCaFlt3L$_{882}$. Insert size and identity were confirmed by restriction digestion, PCR, and sequencing analyses.

Stable transfectants expressing the recombinant molecule pCMV-nCaFlt3L$_{882}$ were established in Chinese Hamster Ovary cells (CHO, available from ATCC) as follows. Briefly, six-well polystyrene tissue culture plates were seeded with approximately 4 x 10^5 cells per well in 2 ml of MEM (available from Life Technologies, Gaithersburg, MD) supplemented with 100 mM L-glutamine, gentamicin, and 10% FBS (TCM). Cells were grown to about 80% confluence (about 18 hr). The recombinant molecule to be transfected was prepared using the Qiagen Endotoxin-Free Plasmid Maxi Kit as per the manufacturer's instructions. The recombinant molecule was linearized with the restriction enzyme *Pvu*I, extracted with phenol, and precipitated with isopropanol. The plasmid pcDNA 3, available from Invitrogen, which contains the neomycin resistance gene, was linearized with the restriction enzyme *Eco*RI . Approximately 1 μg of recombinant plasmid DNA and 100 ng of pcDNA3 were mixed with about 100 μl OptiMEM medium, available from Life Technologies. About 10 μl Lipofectamine (available from Life Technologies) was mixed with 100 μl OptiMEM. The DNA-containing mixture was then added to the Lipofectamine mixture and incubated at room temperature for about 30 min. After incubation, about 800 μl of OptiMEM was added, and the entire mixture was overlaid onto the CHO cells that had been rinsed with OptiMEM. Cells were incubated for 6 hours at 37°C, 5% CO_2, 95% relative humidity. Approximately 1 ml of TCM with 20% FBS was added, and the cells were incubated overnight. The media was changed after about 24 hr. About 72 hr post transfection, the cells were split 1:4 and put into selection TCM containing 500 μg/ml

Geneticin (G418), available from Life Technologies. The medium was changed every 3-5 days. After several weeks, G418-resistant colonies were trypsinized, and the cells were plated into 24 well plates. The resulting recombinant cells are referred to herein as CHO-pCMV-nCaFlt3L$_{882}$. The recombinant cells were then expanded for testing.

iii. The following describes the detection of expression of a canine Flt-3 ligand protein of the present invention by CHO-pCMV-nCaFlt3L$_{882}$, a recombinant cell of the present invention.

Recombinant cells CHO-pCMV-nCaFlt3L$_{882}$, produced as described in Example 2, part (B)(ii) above, were tested for surface expression of canine Flt-3 ligand using a cross-reactive goat anti-human Flt-3 ligand polyclonal antibody as follows. Briefly, 1 x 10^5 CHO-pCMV-nCaFlt3L$_{882}$ cells or CHO-pCMV cells (i.e., cells transfected with an empty vector as described in Example 1) were incubated in phosphate buffered saline (PBS) containing 30% fetal bovine serum (FBS) for about 30 min on ice. The cells were then spun down and treated with the following:

Condition	Primary Incubation	Secondary Incubation
1	PBS	Rabbit (Fab'2) anti sheep (H+L) FITC
2	Goat anti-human Flt3 ligand	Rabbit (Fab'2) anti sheep (H+L) FITC

Goat anti-human Flt3 ligand, available from R and D Systems, Minneapolis, MN was used at about 20µg/ml. Rabbit (Fab'2) anti sheep (H+L) FITC, available from Southern Biotechnology Associates, Inc., was used at about 10 µg/ml. These reagents were diluted in PBS/5%FBS. All incubations were in 50 µl for about 1 hr on ice with 2 washes of PBS/5%FBS in between each incubation. Cells were then analyzed on a flow cytometer

(e.g., MoFlow Desk Top System, available from Cytomation, Ft. Collins, CO) with the fluorescein gate set at 10^1. The results are shown below in Table 4.

Table 4. Expression of canine Flt3 ligand on CHO transfectants.

Cells	% positive Condition 1	Condition 2
CHO-pCMV	1	1
CHO-pCMV nCaFlt3L$_{882}$	2	48
CHO-pCMV nCaFlt3L$_{882}$	1	20

Table 4 shows that canine Flt3 ligand is expressed on the surface of the CHO transfectants.

B. Feline Flt-3 ligand nucleic acid molecules and proteins.

This example describes the production of certain feline Flt-3 ligand nucleic acid molecules and proteins of the present invention.

A nucleic acid molecule encoding a feline Flt 3 ligand was isolated from a feline PBMC cDNA library as follows. A *Felis catus* mitogen activated PBMC cDNA library was constructed in the Uni-Zap-R XR™ vector, available from Stratagene, La Jolla, Ca, using Stratagene's Zap-cDNA-R™ Synthesis Kit and the manufacturer's protocol using mRNA isolated from *F. catus* peripheral blood mononuclear cells about 4 hours after they were activated by a polyclonal activating agent in culture. PCR amplification to isolate a feline Flt 3 ligand nucleic acid molecule was conducted according to the following set of steps: one initial denaturation step at 95°C for 3 minutes; then 35 cycles of the following: 94°C for 30 seconds, 53.8°C for 30 seconds, and 72°C for 105 seconds; then one final extension step at 72°C for 8 minutes. A 395-nucleotide cDNA fragment

containing the 5' end of feline Flt3 ligand coding region, denoted $nFeFlt3L_{395}$, was amplified from the feline PMBC cDNA library using the following primers: vector primer T3 having nucleic acid sequence 5' AATTAACCCT CACTAAAGGG 3' (SEQ ID NO:142) (available from Stratagene) and the antisense primer having SEQ ID NO:14, described in Example 2A. The nucleic acid sequence of the coding strand of $nFeFlt3L_{395}$ is denoted SEQ ID NO:41. A 793-nucleotide cDNA fragment containing the 3' end of feline Flt3 ligand coding region, denoted $nFeFlt3L_{793}$, was isolated using sense primer 2 having the nucleic acid sequence 5' CACAGYCCCA TCTCCTCC 3' (where Y was either T or C) denoted herein as SEQ ID NO:151, in conjunction with vector primer T7 having the nucleic acid sequence 5' GTAATACGAC TCACTATAGG GC 3' (SEQ ID NO:152). The nucleic acid sequence of the coding strand of $nFeFlt3L_{793}$ is denoted SEQ ID NO:42. Nucleic acid molecules $nFeFlt3L_{395}$ and $nFeFlt3L_{793}$ overlap by 246 nucleotides and form a composite sequence encoding a Flt3 ligand protein that is similar in length to that of $PCaFlt3L_{294}$. This composite feline Flt3 ligand cDNA is referred to herein as $nFeFlt3L_{942}$, the coding strand of which was shown to have nucleic acid sequence SEQ ID NO:43. The reverse complement of SEQ ID NO:43 is referred to herein as SEQ ID NO:45. Translation of SEQ ID NO:43 suggests that nucleic acid molecule $nFeFlt3L_{942}$ encodes a Flt3 ligand protein of 291 amino acids, denoted herein as $PFeFlt3L_{291}$, the amino acid sequence of which is presented in SEQ ID NO:44, assuming an open reading frame having an initiation codon spanning from nucleotide 31 through nucleotide 33 of SEQ ID NO:43 and a stop codon spanning from nucleotide 904 through nucleotide 906 of SEQ ID NO:43. The coding region encoding $PFeFlt3L_{291}$, not including the termination codon, is presented herein as $nFeFlt3L_{873}$, which has the

nucleotide sequence SEQ ID NO:46 (the coding strand) and SEQ ID NO:47 (the complementary strand). A putative signal sequence coding region extends from nucleotide 31 to nucleotide 108 of SEQ ID NO:43. The proposed mature protein, denoted herein as PFeFlt3L$_{265}$, denoted SEQ ID NO:49, contains about 265 amino acids, extending from residue 27 though residue 291 of SEQ ID NO:44. The nucleic acid molecule encoding PFeFlt3L$_{265}$ is denoted herein as nFeFlt3L$_{795}$, (SEQ ID NO:48) extending from nucleotide 109 through nucleotide 903 of SEQ ID NO:43. SEQ ID NO:48 has a complementary strand denoted SEQ ID NO:50.

Sequence alignment indicates that nucleic acid sequence SEQ ID NO:43 shares the highest (67.8%) identity with the nucleic acid sequence of human Flt-3 ligand (GenBank accession numbers U04806 and U03858). Amino acid sequence SEQ ID NO:44 shares the highest (70.2%) identity with human Flt-3 ligand protein (GenBank accession numbers U04806 and U03858).

Example 3.

This example describes the isolation and sequencing of certain canine CD40 and feline CD40 nucleic acid molecules and proteins of the present invention.

A. Canine CD40 nucleic acid molecules and proteins

This example describes the production of certain canine CD40 nucleic acid molecules and proteins of the present invention.

A canine CD40 nucleic acid molecule of the present invention was produced by PCR amplification as follows. A 321-nucleotide canine CD40 nucleic acid molecule, denoted nCaCD40$_{321}$, was amplified from a canine PBMC cDNA library, prepared as described in Example 1, using two degenerate oligonucleotide primers designed in

accordance with conserved regions of human, bovine, rabbit, and mouse CD40 gene sequences available in GenBank: sense primer, 5' TGCCCRSTCG GCTTCTTCTC C 3', denoted herein as SEQ ID NO:128; and antisense primer, 5' CGACTCTCTT TRCCRTCCTC CTG 3', denoted herein as SEQ ID NO:129, where R was either A or G and S was either G or C. PCR conditions were as follows: one initial denaturation step at 95°C for 3 minutes; then 35 cycles of the following: 94°C for 30 seconds, then 53°C for 30 seconds, then 72°C for 105 seconds; followed by one final extension at 72°C for 5 minutes. The resulting PCR product, i.e., $nCaCD40_{321}$, with a coding strand represented by SEQ ID NO:51, was radiolabeled using standard techniques and used to screen the canine PBMC cDNA library, under the following hybridization conditions: hybridized in 6X SSC, 5X Denhardt's solution, 0.5% SDS, 100 μg/ml single stranded DNA, 100 μg/ml tRNA for 36 hours at 68°C, followed by a wash of 0.1% SDS, 1X SSC at 55°C for 60 minutes. A clone (Clone 18B) containing a 1425-nucleotide canine CD40 nucleic acid molecule, denoted $nCaCD40_{1425}$, was obtained. The nucleic acid sequence of the coding strand of $nCaCD40_{1425}$ is represented as SEQ ID NO:52. The reverse complement of SEQ ID NO:52 is referred to herein as SEQ ID NO:54. Translation of SEQ ID NO:52 suggests that nucleic acid molecule $nCaCD40_{1425}$ encodes a canine CD40 protein of 274 amino acids, denoted herein as $PCaCD40_{274}$, the amino acid sequence of which is presented in SEQ ID NO:53, assuming an open reading frame having an initiation codon spanning from nucleotide 196 through nucleotide 198 of SEQ ID NO:52 and a stop codon spanning from nucleotide 1018 through nucleotide 1020 of SEQ ID NO:52. The coding region encoding $PCaCD40_{274}$, not including the termination codon, is presented herein as

$nCaCD40_{822}$, which has the nucleotide sequence SEQ ID NO:55 (the coding strand) and SEQ ID NO:56 (the complementary strand).

A putative signal sequence coding region extends from nucleotide 196 through nucleotide 252 of SEQ ID NO:52. The proposed mature protein, denoted herein as $PCaCD40_{255}$, represented by SEQ ID NO:58, contains about 255 amino acids, extending from residue 20 through residue 274 of SEQ ID NO:53. The nucleotide sequence encoding $PCaCD40_{255}$, which extends from nucleotide 253 through nucleotide 1017 of SEQ ID NO:52, is denoted herein as nucleic acid molecule $nCaCD40_{765}$, represented by SEQ ID NO:57 (the coding strand) and SEQ ID NO:59 (the complement strand).

Sequence analysis was performed with DNAsis™ using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10. At the amino acid level, $PCaCD40_{274}$ shares 65.3%, 50.1%, and 42.3% identity with the CD40 proteins of human, bovine, and mouse, respectively (Stamenkovic et al., *EMBO J.,* vol. 8:1403-1410, 1989; Hirano et al., *Immunology,* vol. 90, pp. 294-300, 1997; Grimaldi et al., *J. Immunol.,* vol. 143, pp.3921-3926; Torres and Clark, *J. Immuno.,* vol. 148, pp. 620-626). At the nucleotide level, $nCaCD40_{1425}$ shares 69.3%, 69.4%, and 40.4% identity with the cDNA sequences of human, bovine, and mouse CD40, respectively.

B. Feline CD40 nucleic acid molecules and proteins

This example describes the isolation and sequencing of certain nucleic acid molecules of the present invention that encode certain feline CD40 proteins of the present invention.

A 336-nucleotide feline CD40 nucleic acid molecule, denoted $nFeCD40_{336}$, was amplified from a feline PBMC cDNA library, prepared as described in Example 2, using PCR conditions and primers as described in Example 3A, i.e., a sense primer having SEQ ID NO:128; and an antisense primer having SEQ ID NO:129. The resulting PCR product, i.e., $nFeCD40_{336}$, was shown to have a coding strand the nucleic acid sequence of which is represented as SEQ ID NO:60. The reverse complement of SEQ ID NO:60 is referred to herein as SEQ ID NO:62. Translation of SEQ ID NO:60 suggests that nucleic acid molecule $nFeCD40_{336}$ encodes a partial CD40 protein of 112 amino acids, denoted herein as $PFeCD40_{112}$, the amino acid sequence of which is presented in SEQ ID NO:61, assuming an open reading frame spanning from nucleotide 1 through nucleotide 336 of SEQ ID NO:60.

Comparison of nucleic acid sequence SEQ ID NO:60 with nucleic acid molecules reported in GenBank indicates that SEQ ID NO:60 showed the most homology, i.e. 67.2% identity, with a human CD40 gene. Comparison of amino acid sequence SEQ ID NO:61 with amino acid sequences reported in GenBank indicates that SEQ ID NO:61 showed the most homology, i.e. about 54.4% identity, with a human CD40 protein. Sequence analysis was performed using the GCG GAP program as described above.

Example 4

This example describes the isolation and sequencing of certain canine CD154 (canine CD40 ligand) and feline CD154 (feline CD40 ligand) nucleic acid molecules and proteins of the present invention.

A. Canine CD154 (CD40 ligand) nucleic acid molecules and proteins

The following describes the isolation and sequencing of certain cDNA nucleic acid molecules encoding certain canine CD154 (CD40 ligand) proteins of the present invention.

A canine CD154 nucleic acid molecule of the present invention was produced by PCR amplification as follows. A 390-nucleotide canine CD40 nucleic acid molecule, denoted $nCaCD154_{390}$, was amplified from a canine PBMC cDNA library, prepared as described in Example 1, using two degenerate oligonucleotide primers designed in accordance with human CD154 gene sequences available in GenBank: sense primer, 5' CCTCAAATTG CGGCACATGT C 3', denoted herein as SEQ ID NO:130; and antisense primer, 5' CTGTTCAGAG TTTGAGTAAG CC 3', denoted herein as SEQ ID NO:131. PCR conditions used for canine CD154 cDNA amplification were standard conditions for PCR amplification (Sambrook, et al., *ibid.*). The resulting PCR product, i.e., $nCaCD154_{390}$, with a coding strand represented by SEQ ID NO:63, was radiolabeled using standard techniques and used to screen the canine PBMC cDNA library, under the hybridization conditions described in Example 3. A clone containing a 1878-nucleotide canine CD154 nucleic acid molecule, denoted $nCaCD154_{1878}$, was obtained. The nucleic acid sequence of the coding strand of $nCaCD154_{1878}$ is represented as SEQ ID NO:64. The reverse complement of SEQ ID NO:64 is referred to herein as SEQ ID NO:66. Translation of SEQ ID NO:64 suggests that nucleic acid molecule $nCaCD154_{1878}$ encodes a CD154 protein of 260 amino acids, denoted herein as $PCaCD154_{260}$, the amino acid sequence of which is presented in SEQ ID NO:65, assuming an open reading frame having an initiation codon spanning from nucleotide 284 through nucleotide 286 of SEQ

ID NO:64 and a stop codon spanning from nucleotide 1064 through nucleotide 1066 of SEQ ID NO:64. The coding region encoding $PCaCD154_{260}$, not including the termination codon, is presented herein as $nCaCD154_{780}$, which has the nucleotide sequence SEQ ID NO:67 (the coding strand) and SEQ ID NO:68 (the complementary strand).

A putative signal/membrane anchor sequence coding region extends from nucleotide 284 through nucleotide 430 of SEQ ID NO:64. The proposed soluble CD154 protein, denoted herein as $PCaCD154_{211}$, represented by SEQ ID NO:70, contains about 211 amino acids, extending from residue 50 though residue 260 of SEQ ID NO:65. The nucleotide sequence encoding $PCaCD154_{211}$, which extends from nucleotide 431 through nucleotide 1063 of SEQ ID NO:64, is denoted herein as nucleic acid molecule $nCaCD154_{633}$, represented by SEQ ID NO:69 (the coding strand) and SEQ ID NO:71 (the complement strand).

Sequence analysis was performed with DNAsis™ using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10. At the amino acid level, $PCaCD154_{260}$ shares 78.0%, 77.6%, and 67.6% identity with the CD154 proteins of human, bovine, and mouse, respectively (Graf et al., *Eur. J. Immunol.,* vol. 22, pp. 3191-3194, 1992; Hollenbaugh, et al., *EMBO J.,* vol. 11:4313-4321, 1992; Gauchat et al., *FEBS lett.,* vol., 315, pp. 259-266, 1993; Mertens et al., *Immunogenetics,* vol. 42, pp. 430-431; Armitage et al., *Nature,* vol. 357, pp. 80-82; 1992). At the nucleotide level, $nCaCD154_{1878}$ shares 81.1%, 81.5%, and 74.4% identity with the sequences of human, bovine, and mouse CD154 cDNAs, respectively.

B. Feline CD154 (CD40 ligand) nucleic acid molecules and proteins

This example describes the isolation and sequencing of certain nucleic acid molecules encoding certain feline CD154 (CD40 ligand) proteins of the present invention.

A feline CD154 nucleic acid molecule was isolated by PCR amplification from a feline PBMC cDNA library, prepared as described in Example 2, using Amplitaq DNA polymerase (available from PE Applied Biosystems Inc, Foster City, CA) under the following PCR protocol: one initial denaturation step at 95°C for 5 minutes; then 40 cycles of the following: 94°C for 45 seconds, then 48°C for 45 seconds, then 72°C for 120 seconds; followed by a final extension at 72°C for 7 minutes. The forward and reverse primers used were based on human CD154 cDNA sequences outside the open reading frame in the 5' and 3' untranslated regions, respectively, so that the open reading frame in the PCR product contained only feline sequences. The sequence of the forward primer was 5'GAAGATACCA TTTCAACTTT AACACAGC 3' SEQ ID NO:132, and that of the reverse primer was 5' TGCTGTATTG TGAAGACTCC CAGC 3' SEQ ID NO:133. PCR products were cloned into the TA cloning vector (available from Invitrogen Corporation, Carlsbad, CA), and the resulting clones were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.).

The PCR product was sequenced and found to contain 885 nucleotides, and is denoted as $nFeCD154_{885}$. The nucleotide sequence of the coding strand of $nFeCD154_{885}$

is represented herein as SEQ ID NO:72, and its complement is denoted SEQ ID NO:74. Translation of the open reading frame in SEQ ID NO:72 suggests that $nFeCD154_{885}$ encodes a protein containing 260 amino acids, referred to herein as $PFeCD154_{260}$, the amino acid sequence of which is presented as SEQ ID NO:73, assuming an open reading frame in which the first codon spans from nucleotide 29 through nucleotide 31 of SEQ ID NO:72, and the stop codon spans from nucleotide 809 through nucleotide 811 of SEQ ID NO:72. The encoded protein has a predicted molecular weight of 28.6 kDa for the precursor protein and 27.2 kDa for the mature protein. The coding region encoding $PFeCD154_{260}$, not including the termination codon, is presented herein as $nFeCD154_{780}$, which has the nucleotide sequence SEQ ID NO:75 (the coding strand) and SEQ ID NO:76 (the complementary strand)

A putative signal/membrane anchor sequence coding region extends from nucleotide 29 through nucleotide 175 of SEQ ID NO:72. The proposed soluble feline CD154 protein, denoted herein as $PFeCD154_{211}$, represented by SEQ ID NO:78, contains about 211 amino acids, extending from residue 50 though residue 260 of SEQ ID $NO:_{73}$. The nucleotide sequence encoding $PFeCD154_{211}$, denoted herein as $nFeCD154_{633}$ which extends from nucleotide 176 through nucleotide 808 of SEQ ID NO:72, is represented herein by SEQ ID NO:77 (the coding strand) and SEQ ID NO: 79 (the complementary strand).

Comparison of feline CD154 nucleotide and amino acid sequences with those of other species published in GenBank reveals that the feline CD154 nucleotide sequence SEQ ID NO:75 is 86%, 88% and 75% identical to the human, bovine and murine CD154 gene sequences, respectively (Genbank accession number L07414, Z48469 and X56453

respectively). At the amino acid sequence level, SEQ ID NO:73 is 81%, 82%, and 67% identical to the human, bovine and murine CD154 amino acid sequences, respectively. Hydrophobicity analysis of feline CD154 proteins results in a pattern similar to those of human, bovine and murine CD154 proteins. A putative N-glycosylation site was identified at position 239 in $PFeCD154_{260}$ that is conserved in the human, bovine and murine amino acid sequences. Five cysteine residues are present in the feline CD154 protein sequence SEQ ID NO:73. Four of the five residues, located at positions 72, 84, 177 and 217 of $PFeCD154_{260}$, are conserved in all four species and are likely involved in disulfide bond formation. The cysteine residue located at position 193 of $PFeCD154_{260}$ is present in all but the murine sequence.

Example 5

This example describes the isolation and sequencing of certain canine IL-5 nucleic acid molecules and proteins of the present invention. This example also describes expression of canine IL-5 in a *Pichia* expression system and the bioactivity of such an expressed protein.

A. Isolation and sequencing of canine IL-5 nucleic acid molecules and proteins

A canine IL-5 cDNA nucleic acid molecule encoding a canine IL-5 protein was isolated by PCR amplification from a canine PBMC cDNA library (prepared as described in Example 1) using PCR conditions as described in Example 4B and the following primers. Degenerate oligonucleotide primers were designed in accordance with conserved regions of human and cat IL-5 gene sequences available in GenBank: sense primer, 5' ATGCACTTTC TTTGCC 3', denoted herein as SEQ ID NO:134; antisense

primer 1, 5' CTGGAGGAAA AKACTTCRAT GATTCTGATA TCTGAAATAT AT 3', denoted herein as SEQ ID NO:135; and antisense primer 2, 5' CTGACYCTTK STTGGSCCTC ATTCTCA 3', denoted herein as SEQ ID NO:136, where K was G or T, R was either A or G, S was either G or C, and Y was either T or C.

An about 610-nucleotide canine IL-5 nucleic acid molecule, denoted nCaIL-5_{610}, was obtained using primers having SEQ ID NO:134 and SEQ ID NO:135, respectively. The sequence of the coding strand of nCaIL-5_{610} is represented herein as SEQ ID NO:80. The reverse complement of SEQ ID NO:80 is referred to herein as SEQ ID NO:82. Translation of SEQ ID NO:80 suggests that nucleic acid molecule nCaIL-5_{610} encodes an IL-5 protein of 134 amino acids, denoted herein as PCaIL-5_{134}, the amino acid sequence of which is presented in SEQ ID NO:81, assuming an open reading frame having an initiation codon spanning from nucleotide 29 through nucleotide 31 of SEQ ID NO:80 and a stop codon spanning from nucleotide 431 through nucleotide 433 of SEQ ID NO:80. The coding region encoding PCaIL-13_{134}, not including the termination codon, is presented herein as nCaIL-5_{402}, which has the nucleotide sequence SEQ ID NO:83 (the coding strand) and SEQ ID NO:84 (the complementary strand).

An about 488-nucleotide fragment, denoted herein as nCaIL-5_{488}, isolated by PCR with primers having SEQ ID NO:134 and SEQ ID NO:136, respectively, corresponds to nucleotide 1 through nucleotide 488 of nCaIL-5_{610}.

A putative signal sequence coding region extends from nucleotide 29 through nucleotide 85 of SEQ ID NO:80. The proposed mature protein, denoted herein as PCaIL-5_{115}, represented by SEQ ID NO:86, contains about 115 amino acids, extending from residue 20 though residue 134 of SEQ ID NO:81. The nucleotide sequence encoding

PCaIL-5_{115}, which extends from nucleotide 86 through nucleotide 430 of SEQ ID NO:80, is denoted herein as nucleic acid molecule nCaIL-5_{345}, represented by SEQ ID NO:85 (coding strand) and SEQ ID NO:87 (the complement strand).

Sequence analysis was performed with DNAsis™ using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10. At the amino acid level, PCaIL-5_{134} shared 82.8% and 57.4% identity with feline and human IL-5 proteins, respectively (Padrid et al., *Am. J. Vet. Res.*, vol. 59, pp. 1263-1269, 1998; Azuma et al., *Nucleic Acids Res.*, vol. 14, pp. 9149-9158, 1986). At the nucleotide level, nCaIL-5_{610} shared 81.7% and 75% identity with the cDNA sequences of the feline and human IL-5, respectively.

B. Expression of canine IL-5 in *Pichia*

This example describes the expression in *Pichia* of a canine IL-5 cDNA fragment, namely a canine IL-5 nucleic acid molecule denoted nCaIL-5_{348}, the coding strand of which consists of nucleotides 86-433 of SEQ ID NO:80, and as such, encodes a predicted mature canine IL-5 protein having SEQ ID NO:86. Nucleic acid molecule nCaIL-5_{348}, was PCR amplified from nCaIL-5_{610} using sense primer 5' GGG**CTCGAG**A AAAGATTTGC TGTAGAAAAT CCCATG 3' denoted herein as SEQ ID NO:137, with nucleotides 16-36 corresponding to nucleotides 86-106 of SEQ ID NO:80; and antisense primer 5' CCC**GCGGCCG C**TCAACTTTC CGGTGTCCAC TC 3', denoted herein as SEQ ID NO:138, with nucleotides 12-32 corresponding to the reverse complement of nucleotides 413-433 of SEQ ID NO:80. To facilitate cloning, an *Xho*I site (shown in

bold) was added to the sense primer and a *Not*I site (shown in bold) was added to the antisense primer. The PCR-amplified fragment was digested with restriction endonucleases *Xho*I and *Not*I, gel purified and ligated into pPICZαA plasmid vector, available from Invitrogen, that had been digested by *Xho* I and *Not* I and gel purified, to produce recombinant molecule pPICZαA-nCaIL-5_{348}. The insert in the recombinant molecule was verified by DNA sequencing The recombinant molecule was used to transform *Pichia pastoris* strain X-33 by electroporation to produce recombinant cell *Pichia*-pPICZαA-nCaIL-5_{348}. Recombinant cell *Pichia*-pPICZαA-nCaIL-5_{348} was cultured using techniques known to those skilled in the art and IL-5 expression was induced with methanol. The supernatant was recovered and submitted to SDS-PAGE. Silver staining of the resultant gel indicated a band of about 18 kDa only seen in the supernatant of *Pichia* transformed with recombinant molecule pPICZαA-nCaIL-5_{348}.

C. Bioactivity of *Pichia*-expressed canine IL-5

The following describes a bioassay to detect the expression of canine IL-5 by stimulating the proliferation of TF-1 cells.

TF-1 cells, grown and maintained as described in Example 1E, were extensively washed to remove rhuGM-CSF, and then added at approximately 1 x 10^4 cells per well to 96-well flat bottom plates. *Pichia*-expressed canine IL-5, produced as described in Example 5B, was dialyzed overnight at 4° C against Phosphate Buffered Saline, diluted to the appropriate concentration inTCM-TF-1 without rhuGM-CSF and filter sterilized. Cells and *Pichia*-produced canine IL-5 were incubated for 48 hours in 5% CO_2 at 37°C, then pulsed, incubated, harvested and counted as described in Example 1E. The results are shown in Table 5.

Table 5. Stimulation of proliferation of TF-1 with *Pichia*-expressed canine IL-5

1/dilution	Counts per minute
2	44,885
4	101,564
8	81,161
16	59,384
32	40,508
64	15,948
128	6,634
256	2,441
Media (no IL-5)	172

Table 5 shows that canine IL-5 expressed by *Pichia* is biologically active, as demonstrated by its ability to stimulate proliferation of TF-1 cells.

Example 6

This example describes the isolation and sequencing of certain canine IL-13 nucleic acid molecules and proteins of the present invention. This example also describes expression of canine IL-13 in *E. coli* and bioactivity of such an expressed protein.

A. Isolation and sequencing of canine IL-13 nucleic acid molecules and proteins

A canine IL-13 cDNA nucleic acid molecule encoding a canine IL-13 protein was isolated by PCR amplification from a canine PBMC cDNA library (prepared as described in Example 1) using the following primers and PCR conditions: Degenerate oligonucleotide primers were designed in accordance with conserved regions of human and cat IL-5 gene sequences available in GenBank: sense primer, 5' GTCMTKGCTC TYRCTTGCCT TGG 3', denoted herein as SEQ ID NO:139; antisense primer 1, 5' AAASTGGGCY ACYTCGATTT TGG 3', denoted herein as SEQ ID NO:140; antisense primer 2, 5' GTGATGTTGM YCAGCTCCTC 3', denoted herein as SEQ ID

NO:141, where M was either A or C, K was G or T, R was either A or G, S was either G or C, and Y was either T or C. PCR conditions used were as follows: One initial denaturation step at 95°C for 3 minutes; then 38 cycles of the following: 94°C for 30 seconds, 51.8°C for 45 seconds, then 72°C for 105 seconds; then a final extension at 72°C for 5 minutes.

An about 272-nucleotide canine IL-13 nucleic acid molecule, denoted nCaIL-13_{272} and having a coding strand represented by SEQ ID NO:89, was PCR amplified using primers having nucleic acid sequences of SEQ ID NO:139 and SEQ ID NO:140, respectively. An about 166-nucleotide canine IL-13 nucleic acid molecule, denoted nCaIL-13_{166} and having a coding strand represented by SEQ ID NO:88, was isolated using primers having nucleic acid sequences of SEQ ID NO:142 (see Example 2B) and SEQ ID NO:141, respectively. Nucleic acid molecules nCaIL-13_{272} and nCaIL-13_{272} form a overlapping composite fragment of 383 nucleotides, denoted nCaIL-13_{383}. Two canine IL-13 specific primers (i.e., sense primer, 5' ATGGCGCTCT GGTTGACTGT 3', denoted herein as SEQ ID NO:143; and antisense primer, 5' GGCTTTTGAG AGCACAGTGC 3', denoted herein as SEQ ID NO:144) were derived from nCaIL-13_{383} and were used to isolate a 278-nucleotide fragment, denoted nCaIL-13_{278} and having a coding strand represented by SEQ ID NO:90. Nucleic acid molecule nCaIL-13_{278} was radiolabeled and used to screen the canine PBMC cDNA library under the following hybridization conditions: hybridization took place in 6X SSC, 5X Denhardt's solution, 0.5% SDS, 100 μg/ml single stranded DNA, 100 μg/ml tRNA, for 36 hours at 60°C; the final wash solution was 0.1% SDS, 0.125X SSC at 60°C for 30 minutes. Two clones were selected, namely clone 80 and clone 78.

Sequence analysis of Clone 80 indicated that the clone includes an about 1302-nucleotide canine IL-13 nucleic acid molecule referred to herein as nCaIL-13_{1302}, the coding strand of which was shown to have nucleic acid sequence SEQ ID NO:91. The reverse complement of SEQ ID NO:91 is referred to herein as SEQ ID NO:93.

Translation of SEQ ID NO:91 suggests that nucleic acid molecule n nCaIL-13_{1302} encodes an IL-13 protein of 131 amino acids, denoted herein as PCaIL-13_{131}, the amino acid sequence of which is presented in SEQ ID NO:92, assuming an open reading frame having an initiation codon spanning from nucleotide 52 through nucleotide 54 of SEQ ID NO:91 and a stop codon spanning from nucleotide 445 through nucleotide 447 of SEQ ID NO:91. The coding region encoding PCaIL-13_{131}, not including the termination codon, is presented herein as nCaIL-13_{393}, which has the nucleotide sequence SEQ ID NO:94 (the coding strand) and SEQ ID NO:95 (the complementary strand).

A putative signal sequence coding region extends from nucleotide 52 to nucleotide 111 of SEQ ID NO:91. The proposed mature protein, denoted herein as PCaIL-13_{111}, represented by SEQ ID NO:97, contains 111 amino acids, extending from residue 21 through residue 131 of SEQ ID NO:91. The nucleotide sequence encoding PCaIL-13_{111}, which extends from nucleotide 112 through nucleotide 444 of SEQ ID NO:91, is denoted herein as nucleic acid molecule nCaIL-13_{333}, represented by SEQ ID NO:96 (coding strand) and SEQ ID NO:98 (the complement strand).

Sequence analysis of Clone 78 indicated that the clone includes an about 1269-nucleotide canine IL-13 nucleic acid molecule referred to herein as nCaIL-13_{1269}, the coding strand of which was shown to have nucleic acid sequence SEQ ID NO:99. The reverse complement of SEQ ID NO:99 is referred to herein as SEQ ID NO:101.

Translation of SEQ ID NO:99 suggests that nucleic acid molecule nCaIL-13_{1269} encodes an IL-13 protein of 130 amino acids, denoted herein as PCaIL-13_{130}, the amino acid sequence of which is presented in SEQ ID NO:100, assuming an open reading frame having an initiation codon spanning from nucleotide 57 through nucleotide 59 of SEQ ID NO:99 and a stop codon spanning from nucleotide 447 through nucleotide 449 of SEQ ID NO:99. The coding region encoding PCaIL-13_{130}, not including the termination codon, is represented herein as nCaIL-13_{390}, which has the nucleotide sequence SEQ ID NO:102 (the coding strand) and SEQ ID NO:103 (the complementary strand). PCaIL-13_{130} is missing one amino acid compared to PCaIL-13_{133}, namely amino acid position Q97 of PCaIL-13_{133}.

A putative signal sequence coding region extends from nucleotide 57 to nucleotide 116 of SEQ ID NO:99. The proposed mature protein, denoted herein as PCaIL-13_{110}, represented by SEQ ID NO:105, contains 110 amino acids, extending from residue 21 though residue 130 of SEQ ID NO:100. The nucleotide sequence encoding PCaIL-13_{110}, which extends from nucleotide 117 through nucleotide 446 of SEQ ID NO:99, is denoted herein as nucleic acid molecule nCaIL-13_{330}, represented by SEQ ID NO:104 (coding strand) and SEQ ID NO:106 (the complement strand).

Sequence analysis was performed with DNAsis™ using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10. At the amino acid level, PCaIL-13_{131} shared 61.7%, 39.6%, 36.6% identity with the IL-13 proteins of human, mouse, and rat (Brown et al., *J. Immunol.,* vol. 142, pp. 679-687, 1989; Lakkis et al., *Biochem. Biophys. Res. Commun.,* Vol. 197, pp. 612-618, 1993; McKenzie et al., *Proc.*

Natl Acad. Sci. USA, vol. 90, pp. 3735-3739, 1993; Minty et al., *Nature,* vol. 362, pp. 248-250, 1993), respectively. At the nucleotide level, nCaIL-13_{1302} shared 56.0%, 57.1%, and 45.9% identity with the sequences of human, rat, and mouse IL-13 cDNAs, respectively.

B. Expression of canine IL-13 in *E. coli*

This example describes the expression in *E. coli* of a canine IL-13 cDNA fragment, namely a canine IL-13 nucleic acid molecule denoted nCaIL-13_{336}, the coding strand of which consists of nucleotides 112-447 of SEQ ID NO:91, and as such, encodes a predicted mature canine IL-13 protein having SEQ ID NO:97. Nucleic acid molecule nCaIL-13_{336} was PCR amplified from nCaIL-13_{1302} using sense primer 5' CCC**CATATG**A GCCCTGTGAC TCCCTCCCC 3' denoted herein as SEQ ID:145, with nucleotides 10-29 corresponding to nucleotides 112-1131 of SEQ ID NO:91; and antisense primer 5' GGG**GAATTC**T CATCTGAAAT TTCCATGGCG 3', denoted herein as SEQ ID NO:146, with nucleotides 10-30 corresponding to the reverse complement of nucleotides 427-447 of SEQ ID NO:91. To facilitate cloning, an *Nde*I site (shown in bold) was added to the sense primer and an *Eco*RI site (shown in bold) was added to the antisense primer. The resulting PCR fragment was digested with restriction endonucleases *Nde*I and *Eco*RI, gel purified and ligated into λcro plasmid vector, the production of which is described in U.S. Patent No. 5,569,603 by Tripp et al., issued October 29, 1996, that had been digested by *Nde*I and *Eco*RI and gel purified to produce recombinant molecule pλcro-nCaIL-13_{336}. The insert in the recombinant molecule was verified by DNA sequencing. Recombinant molecule pλcro-nCaIL-13_{336} was used to

transform *E. coli* strain HCE101 (BL21), thereby producing BL21-pλcro-nCaIL-13_{336}. PCaIL-13_{111} was produced under conditions as described in U.S. Patent No. 5,569,603, *ibid.*, protein expression being induced by switching the fermentation temperature from 32°C to 42°C. SDS-PAGE and Commassie blue staining analysis indicated that a band of about 11 kD was only produced by induced BL21-pλcro-nCaIL-13_{336} recombinant cells. The 11-kD band showed a positive reaction with a rabbit polyclonal antibody against human IL-13 (available from PeproTech Inc, Rocky Hill, NJ), indicating expression of canine IL-13 in *E. coli*.

C. Bioactivity of *E. coli*-expressed canine IL-13

The following describes a bioassay to detect the expression of canine IL-13 by stimulating the proliferation of TF-1 cells.

TF-1 cells, grown and maintained as described in Example 1E, were extensively washed to remove rhuGM-CSF, and then added at approximately 1 x 10^4 cells per well to 96-well flat bottom plates. *E. coli*-produced PCaIL-13_{111}, produced as described in Example 6B, was dialyzed overnight at 4° C against Phosphate Buffered Saline, diluted to the appropriate concentration inTCM-TF-1 without rhuGM-CSF and filter sterilized. Cells and *E. coli*-produced PCaIL-13_{111} were incubated for 48 hours in 5% CO_2 at 37°C, then pulsed, incubated, harvested and counted as described in Example 1E. The results are shown in Table 6.

Table 6. Stimulation of proliferation of TF-1 with *E. coli* PCaIL-13_{111}

Concentration *E. coli* PCaIL-13_{111} (ng/ml)	Counts per minute
1000	126,203
500	77,893
250	57,781
125	40,491
62.5	26,115
31.3	7,042
15.6	8,713
0	991

Table 6 shows that canine IL-13 expressed by *E. coli* is biologically active, as demonstrated by its ability to stimulate proliferation of TF-1 cells.

Example 7

This example describes the isolation and sequencing of feline interferon alpha nucleic acid molecules and proteins of the present invention. This example also describes expression of feline interferon alpha proteins of the present invention in *E. coli* and mammalian cells as well as the bioactivities of the resulting proteins.

A. Isolation and sequencing of feline IFN-alpha nucleic acids and proteins

Feline IFN-alpha nucleic acid molecules were PCR amplified from a feline cDNA library as follows. Total RNA was isolated from cat (kitten) mesenteric lymph node cells stimulated with PMA (phorbol myristate acetate) for 48 hours using Tri Reagent ™ (available from Molecular Research Center, Cincinnati, Ohio). cDNA was made from the RNA using the cDNA synthesis kit containing Ready to Go -You Prime First-Strand Beads™ (available from Amersham Pharmacia Biotech, Piscataway, NJ). An aliquot of this cDNA was used as a template to isolate a feline IFN-alpha nucleic acid molecule by PCR amplification using Amplitaq DNA polymerase ™ (available from PE Applied

Biosystems Inc, Foster City, CA) and the following primers and conditions. The sequence of the forward primer was 5'ATGGCGCTGC CCTCTTCCTT CTTG 3' (SEQ ID NO:143), and that of the reverse primer was 5' TCATTTCTCG CTCCTTAATC TTTTCTGC 3' (SEQ ID NO:148). The following PCR protocol was used: one initial denaturation step at 95°C for 5 minutes; then 43 cycles of the following: 94°C for 45 seconds, then 47°C for 45 seconds, then 72°C for 120 seconds; followed by a final extension at 72°C for 7 minutes. PCR products were cloned into the TA cloning vector (available from Invitrogen Corporation) and the clones were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). Five PCR products were generated and sequenced. These products were included, respectively, in Clones #1, #2, #3, #5, and #6.

Clone #2 includes a feline IFN-alpha nucleic acid molecule that is represented herein as nFeIFNα_{567a}, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:107. The complement of SEQ ID NO:107 is represented herein by SEQ ID NO:109. Translation of SEQ ID NO:107 suggests that nFeIFNα_{567a} encodes a protein containing 189 amino acids, referred to herein as PFeIFNα_{189a}, with an amino acid sequence denoted SEQ ID NO:108. The open reading frame of SEQ ID NO:107 is assumed to be the following: the first codon spans from nucleotide 1 through nucleotide 3 and the last codon before the stop codon spans from nucleotide 565 to nucleotide 567 of SEQ ID NO:107. The encoded protein has a predicted molecular weight of 21 kDa. The putative signal peptide cleavage site occurs

between amino acid positions 23 and 24, based on homology with the human and canine interferon-alpha proteins. The proposed mature protein (i.e. feline IFNα protein from which the signal sequence has been cleaved), denoted herein as PFeIFNα$_{166a}$, contains about 166 amino acids, extending from residue 24 to residue 166 of SEQ ID NO:108; the amino acid sequence is denoted herein as SEQ ID NO:114. The nucleic acid molecule encoding PFeIFNα$_{166a}$ is denoted herein as nFeIFNα$_{498a}$, the coding strand of which is represented by SEQ ID NO:113, and the complementary strand of which is represented by SEQ ID NO:115. A putative N-glycosylation site and an interferon alpha-beta-delta family signature motif are present at amino acid positions 102 and 145, respectively, of PfeIFNα$_{189a}$.

Clone #3 includes a feline IFN-alpha nucleic acid molecule that is represented herein as nFeIFNα$_{567b}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:110. The complement of SEQ ID NO:110 is represented herein by SEQ ID NO:112. Translation of SEQ ID NO:110 suggests that nFeIFNα$_{567b}$ encodes a protein containing 189 amino acids, referred to herein as PFeIFNα$_{189b}$, with an amino acid sequence denoted SEQ ID NO:111. The open reading frame of SEQ ID NO:110 is assumed to be the following: the first codon spans from nucleotide 1 through nucleotide 3 and the last codon before the stop codon spans from nucleotide 565 through nucleotide 567 of SEQ ID NO:110. The encoded protein has a predicted molecular weight of 21 kDa. The putative signal peptide cleavage site occurs between amino acid positions 23 and 24, based on homology with the human and canine interferon-alpha proteins. The proposed mature protein (i.e. feline IFNα protein from which the signal sequence has been cleaved), denoted herein as PFeIFNα$_{166b}$, contains

about 166 amino acids, extending from residue 24 to residue 166 of SEQ ID NO:111; the amino acid sequence is denoted herein as SEQ ID NO:117. The nucleic acid molecule encoding PFeIFNα_{166b} is denoted herein as nFeIFNα_{498b}, the coding strand of which is represented by SEQ ID NO:116, and complementary strand of which is represented by SEQ ID NO:118. A putative N-glycosylation site and an interferon alpha-beta-delta family signature motif are present at amino acid positions 102 and 145, respectively, of PFeIFNα_{189b}.

Clone #1 includes a feline IFN-alpha nucleic acid molecule that is represented herein as nFeIFNa$_{567c}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:155. The complement of SEQ ID NO:155 is represented herein by SEQ ID NO:157. Translation of SEQ ID NO:155 suggests that nFeIFNa$_{567c}$ encodes a protein containing 189 amino acids, referred to herein as PFeIFNa$_{189c}$, with an amino acid sequence denoted SEQ ID NO:156. The open reading frame of SEQ ID NO:155 is assumed to be the following: the first codon spans from nucleotide 1 through nucleotide 3 and the last codon before the stop codon spans from nucleotide 565 to nucleotide 567 of SEQ ID NO:155. The encoded protein has a predicted molecular weight of 21 kDa. The putative signal peptide cleavage site occurs between amino acid positions 23 and 24, based on homology with the human and canine interferon-alpha proteins. The proposed mature protein (i.e. feline IFNa protein from which the signal sequence has been cleaved), denoted herein as PFeIFNa$_{166c}$, contains about 166 amino acids, extending from residue 24 to residue 166 of SEQ ID NO:156; the amino acid sequence is denoted herein as SEQ ID NO:159. The nucleic acid molecule encoding PFeIFNa$_{166c}$ is denoted herein as nFeIFNa$_{498c}$, the coding strand of which is

represented by SEQ ID NO:158, and the complementary strand of which is represented by SEQ ID NO:160. A putative N-glycosylation site and an interferon alpha-beta-delta family signature motif are present at amino acid positions 102 and 145, respectively, of $PfeIFNa_{189c}$.

Clone #5 includes a feline IFN-alpha nucleic acid molecule that is represented herein as $nFeIFNa_{582d}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:161. The complement of SEQ ID NO:161 is represented herein by SEQ ID NO:163. Translation of SEQ ID NO:161 suggests that $nFeIFNa_{582d}$ encodes a protein containing 194 amino acids, referred to herein as $PFeIFNa_{194d}$, with an amino acid sequence denoted SEQ ID NO:162. The open reading frame of SEQ ID NO:161 is assumed to be the following: the first codon spans from nucleotide 1 through nucleotide 3 and the last codon before the stop codon spans from nucleotide 580 through nucleotide 582 of SEQ ID NO:161. The encoded protein has a predicted molecular weight of 21.5 kDa. The putative signal peptide cleavage site occurs between amino acid positions 23 and 24, based on homology with the human and canine interferon-alpha proteins. The proposed mature protein (i.e. feline IFNa protein from which the signal sequence has been cleaved), denoted herein as $PFeIFNa_{171d}$, contains about 171 amino acids, extending from residue 24 to residue 171 of SEQ ID NO:162; the amino acid sequence is denoted herein as SEQ ID NO:165. The nucleic acid molecule encoding $PFeIFNa_{171d}$ is denoted herein as $nFeIFNa_{513d}$, the coding strand of which is represented by SEQ ID NO:164, and the complementary strand of which is represented by SEQ ID NO:166. A putative N-glycosylation site and an interferon alpha-beta-delta

family signature motif are present at amino acid positions 102 and 145, respectively, of $PFeIFNa_{194d}$.

Clone #6 includes a feline IFN-alpha nucleic acid molecule that is represented herein as $nFeIFNa_{567e}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:167. The complement of SEQ ID NO:167 is represented herein by SEQ ID NO:169. Translation of SEQ ID NO:167 suggests that $nFeIFNa_{567e}$ encodes a protein containing 189 amino acids, referred to herein as $PFeIFNa_{189e}$, with an amino acid sequence denoted SEQ ID NO:168. The open reading frame of SEQ ID NO:167 is assumed to be the following: the first codon spans from nucleotide 1 through nucleotide 3 and the last codon before the stop codon spans from nucleotide 565 to nucleotide 567 of SEQ ID NO:167. The encoded protein has a predicted molecular weight of 21 kDa. The putative signal peptide cleavage site occurs between amino acid positions 23 and 24, based on homology with the human and canine interferon-alpha proteins. The proposed mature protein (i.e. feline IFNa protein from which the signal sequence has been cleaved), denoted herein as $PFeIFNa_{166e}$, contains about 166 amino acids, extending from residue 24 to residue 166 of SEQ ID NO:167; the amino acid sequence is denoted herein as SEQ ID NO:171. The nucleic acid molecule encoding $PFeIFNa_{166e}$ is denoted herein as $nFeIFNa_{498e}$, the coding strand of which is represented by SEQ ID NO:170, and the complementary strand of which is represented by SEQ ID NO:172. A putative N-glycosylation site and an interferon alpha-beta-delta family signature motif are present at amino acid positions 102 and 145, respectively, of $PfeIFNa_{189e}$.

Comparison of the nucleic acid sequences of the five feline IFN-alpha nucleic acid molecules of the present invention indicated that the sequences, while being very similar (i.e., encoded proteins sharing from about 96% to 99% identity), exhibited several differences. The differences in nucleic acid sequences and deduced amino acid sequences are summarized in Table 7. The left hand column indicates the change at the nucleotide or amino acid level, as appropriate, and the "X"s in the other columns indicate which clones include such changes. For example, feline IFN-alpha protein $PfeIFNa_{194d}$ (having SEQ ID NO:161) has five extra amino acids (namely IHPED) inserted at position at 139 as compared to feline IFN-alpha proteins $PfeIFNa_{189a}$ (SEQ ID NO:108), $PfeIFNa_{189b}$ (SEQ ID NO:111), $PfeIFNa_{189c}$ (SEQ ID NO:155) or $PfeIFNa_{189e}$ (SEQ ID NO:167). Other variations, i.e., nucleotide substitutions, some of which lead to amino acid variations, are also indicated in Table 7.

Table 7. Comparison of feline IFN-alpha nucleic acid molecules and proteins

Amino acid Changes	Clone # 1	Clone # 2	Clone # 3	Clone # 5	Clone # 6
5 amino acid deletion	X	X	X		X
S_{18} to S_{18} (TCC to TCT)					X
C_{52} to C_{52} (TGT to TGC)					X
R_{56} to R_{56} (AGA to AGG)			X		
N_{57} to S_{57} (AAT to AGT)	X			X	
F_{66} to F_{66} (TTC to TTT)	X	X			
A_{74} to A_{74} (GCC to GCT)			X		
K_{86} to E_{86} (AAG to GAG)			X		
R_{115} to W_{115} (CGG to TGG)	X	X			
L_{125} to V_{125} (CTG to GTG)			X	X	X
L_{125} to M_{125} (CTG to ATG)	X	X			
L_{135} to L_{135} (CTG to CTC)	X	X	X		X
I_{141} to L_{141} (ATC to CTC)			X		

Feline IFN-alpha proteins of the present invention PFeIFNα_{189a}, PfeIFNα_{189b}, PFeIFNα_{189c}, and PFeIFNα_{189e} are five amino acids shorter than the GenBank entry for feline IFN-omega, accession # E02521, while IFN-alpha protein PFeIFNα_{194d} of the present invention has the same number of amino acids as the feline IFN-omega reported in GenBank. In addition, there are: 3 non-conservative and 2 conservative changes at the amino acid level between this GenBank entry and SEQ ID NO:108 (PFeIFNα_{189a}); 4 non-conservative and 3 conservative changes at the amino acid level between this GenBank

entry and SEQ ID NO:111 (PfeIFNα_{189b}); 4 non-conservative and 3 conservative changes at the amino acid level between this GenBank entry and SEQ ID NO:156 (PFeIFNa$_{189c}$); 2 non-conservative and 2 conservative changes at the amino acid level between this GenBank entry and SEQ ID NO:162 (PfeIFNa$_{194d}$; and 1 non-conservative and 5 conservative changes at the amino acid level between this GenBank entry and SEQ ID NO:168 (PFeIFNa$_{189e}$).

The lengths of SEQ ID NO:108 and SEQ ID NO:111, when compared with those of IFN-alpha proteins of other species, are two amino acids longer than published canine interferon-alpha subtype 1, 2 and 3 sequences, two amino acids longer than published human interferon-alpha type 1,B,D, F, and J sequences, three amino acids longer than the published human interferon-alpha sequence type A sequence and two amino acids longer than published murine interferon-alpha type B, 8, 7, 11, and 19 sequences. The lengths of SEQ ID NO:156 and SEQ ID NO:168, when compared with those of IFN-alpha proteins of other species, are two amino acids longer than published canine interferon-alpha subtype 1, 2 and 3 sequences, two amino acids longer than published human interferon-alpha type 1,B,D, F, and J sequences, three amino acids longer than the published human interferon-alpha sequence type A sequence and two amino acids longer than published murine interferon-alpha type B, 8, 7, 11, and 19 sequences. The length of SEQ ID NO:162, when compared with those of IFN-alpha proteins of other species, are seven amino acids longer than published canine interferon-alpha subtype 1, 2 and 3 sequences, seven amino acids longer than published human interferon-alpha type 1,B,D, F, and J sequences, eight amino acids longer than the published human interferon-alpha sequence

type A sequence and seven amino acids longer than published murine interferon-alpha type B, 8, 7, 11, and 19 sequences.

B. Expression of feline IFN-alpha proteins in mammalian cells

This example describes the expression of the feline IFN-alpha proteins of the present invention in Chinese hamster ovary (CHO) cells.

Feline IFN-alpha nucleic acid molecule PCR products were amplified from $nFeIFN\alpha_{567a}$,$nFeIFN\alpha_{567b}$, $nFeIFN\alpha_{567c}$, $nFeIFN\alpha_{582d}$, and $nFeIFN\alpha_{567e}$ using Pfu DNA polymerase™ (available from Stratagene, La Jolla, CA) and the following primers and conditions. The sequence of the forward primer was 5'ATTAGGATCC ATGGCGCTGC CCTCTTCCT 3' (SEQ ID NO:173), and that of the reverse primer was 5'GCCTCTAGAC TGTCATTTCT CGCTCCTTAA TCTTTTCTGC 3' (SEQ ID NO:174). The following PCR protocol was used: one initial denaturation step at 95°C for 5 minutes; then 30 cycles of the following: 94°C for 30 seconds, then 50°C for 30 seconds, then 72°C for 90 seconds; followed by a final extension at 72°C for 7 minutes.

Each of the five PCR products was ligated into a CMV-Int A-kan+(amp) expression vector using techniques similar to those described in Example 1Bii to produce recombinant molecules in which feline IFN-alpha nucleic acid molecules were operatively linked to transcription control sequences. It is to be noted that CMV-Int A-kan^+(amp) vector is similar to the pCMV-Int A plasmid vector described in Example 1Bii except that the ampicillin resistance gene open reading frame has been disrupted by the insertion of the kanamycin resistance gene. The feline IFN-alpha nucleic acid molecules in the recombinant molecules were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA

sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). The sequence data indicated that there was no changes introduced during the PCR amplification or ligation in any of the nucleic acid molecules.

Using techniques similar to those described elsewhere herein, CHO cells were transiently transfected with each of the five recombinant molecules encoding a subtype of feline IFN-alpha protein using Lipofectamine™ (available from Life technologies, Inc.) resulting in recombinant cells expressing feline IFN-alpha subtype proteins of the present invention. The cells and culture supernatants were harvested 48 hours later and Western analysis was done using both pellets and the supernatants from each transfection. The detecting antibody was an anti-human IFN-alpha-A antibody (available from Accurate Chemical and Scientific Corporation, Westbury, NY). The Western analysis indicated that each of the five feline IFN-alpha nucleic acid molecule-containing recombinant cells expressed a corresponding feline IFN-alpha subtype protein which was secreted into the tissue culture supernatant and recognized by the antibody against human IFN-alpha-A. The migration patterns of each of the CHO cell-expressed feline IFN-alpha subtype proteins suggested that each of the proteins is glycosylated.

C. Bioactivity of mammalian-expressed feline-IFN alpha proteins

(i) The antiviral activity of the five CHO-expressed feline IFN-alpha subtype proteins, produced as described in Example 7B, was tested using the following protocol: Crandell feline kidney (CRFK) cells were treated for 24 hours, using procedures known to those skilled in the art, with or without IFN-alpha tissue culture supernatants, produced as described in Example 7B. The cells were then infected with feline calicivirus and

cytopathic effects induced by the virus were assessed 12 to 14 hours later using techniques known to those skilled in the art. The cell layers were fixed in methanol, stained with crystal violet and examined under the microscope or processed for the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The MTT assay was conducted as follows. After viral infection, the infected cells were washed with PBS. A volume of MTT stock solution (5 mg/ml in PBS) equal to one-tenth of the original culture volume was added to each well being assayed and incubated at 37oC for 3 to 4 hr. The MTT solution was removed, and acidified isopropanol ().1 N HCl in absolute isopropanol) was added to the wells to solubilize the converted dye. The absorbance of the converted dye was measured at 570 nm using a plate reader. Each of the five IFN-alpha subtype proteins demonstrated anti-viral activity. Pre-treatment with any of the subtypes of IFN-alpha proteins of the present invention resulted in significant reduction in the virus-induced cytopathic effect.

(ii) The CHO cell-expressed feline IFN-alpha subtype proteins were also tested for their ability to inhibit granulocyte-macrophage colony stimulating factor-induced proliferation of TF-1 cells using an assay similar to that described in Example 1E, but with the following modification:. For the assay, the cells were washed and TCM-TF-1 medium containing a suboptimal amount of GM-CSF (i.e., 0.4 ng/ml) was added along with the appropriate dilutions of the designated IFN-alpha proteins. The results are shown in Table 8 for feline IFN-alpha proteins expressed as described in Example 7B, lanes labeled Clone #1, Clone #2, Clone #3, Clone #5 and Clone #6, respectively; supernatant from a culture of CHO cells transfected with only the vector described in Example 7B, lane labeled vector; *E. coli*-expressed feline IFN-alpha protein $PFeIFNa_{166e}$

produced as described in Example 7D, lane labeled *E.coli*-expressed; and recombinant human IFN-alpha, lane labeled human IFN-alpha. Media alone gave a reading of 128 and recombinant GM-CSF alone gave a reading of 96080.

Table 8. Inhibition of TF-1 cell production by CHO cell-expressed feline IFN-alpha proteins

Dilution	Clone #1	Clone #2	Clone #3	Clone #5	Clone #6	Vector	E. coli expressed	Human IFN alpha
2	15077	7914	21173	15218	13256	53585	19541	559
4	18318	23515	41488	43449	31618	64722	56315	10412
8	22484	25823	48487	40438	43896	83092	80646	21710
16	42138	34274	72145	66266	48775	102423	97255	23585
32	81248	52847	63264	95256				
64	74613	43848	58533	88172	70596	141821	129556	45907
128	59360	48901	48701	54623	90092	155960	151946	40402
256	75788	54017	37391	59849	83022	119491	123794	39299

Table 8 demonstrates that CHO cell-expressed and *E. coli*-expressed feline IFN-alpha subtype proteins inhibited granulocyte-macrophage colony stimulating factor-induced proliferation of TF-1 cells.

D. Expression of feline IFN-alpha in *E. coli* and bioactivity thereof

The nucleic acid molecule encoding the mature feline IFN-alpha protein having SEQ ID NO:171 was ligated into the λcro plasmid vector, using techniques as described in Example 6B, to produce recombinant molecule pλcro-nFeIFNa$_{498e}$. The recombinant molecule was transformed into *E. coli*, using techniques similar to those described in Example 6B to produce recombinant cell *E. coli*:pλcro-nFeIFNa$_{498e}$. The recombinant cell was grown and induced as described in Example 6B. The resulting feline IFN-alpha protein, *E. coli*-expressed PFeIFNa$_{166e}$. which was expressed as an insoluble form, was

solubilized using urea and DTT and refolded using techniques known to those skilled in the art. The refolded *E. coli*-expressed feline IFN-alpha protein $PFeIFNa_{166e}$ when tested for antiviral activity as described in Example 7C was found to have significant antiviral activity.

Example 8

This example describes the isolation and sequencing of feline granulocyte-macrophage colony-stimulating factor (GMCSF) nucleic acid molecules and proteins of the present invention. This example also describes expression of a feline GMCSF protein of the present invention.

Nucleic acid molecules encoding feline GMCSF were isolated as follows. A cDNA library was prepared from feline PBMCs stimulated with Con A for 12 hours, as previously described in Example 2. An aliquot of this library was used as a template to amplify feline GMCSF nucleic acid molecules by PCR using Amplitaq DNA polymerase ™ (PE Applied Biosystems Inc, Foster City, CA) and the following primers and conditions The sequence of the forward primer was 5'CAGGGATCCA CCATGTGGCT GCAGAACCTG CTTTTCC 3' (SEQ ID NO:149), and that of the reverse primer was 5' TTACTTCTGG TCTGGTCCCC AGCAGTCAAA GGGGTTGTTA AACAGAAAAT 3' (SEQ ID NO:150). The following PCR protocol was used: one initial denaturation step at 95°C for 5 minutes; then 35 cycles of the following: 94°C for 30 seconds, then 50°C for 30 seconds, then 72°C for 90 seconds; followed by a final extension at 72°C for 7 minutes. PCR products were cloned into the CMV-Intron A vector and the clones were sequenced as described in Example 7.

A PCR product was isolated, referred to herein as $nFeGMCSF_{444}$, the coding strand of which is represented herein as SEQ ID NO:119, and its complement is denoted SEQ ID NO:121. Translation of the open reading frame in SEQ ID NO:119 suggests that nucleic acid molecule $nFeGMCSF_{444}$ encodes a protein containing 144 amino acids, referred to herein as $PFeGMCSF_{144}$, with an amino acid sequence denoted SEQ ID NO:120, assuming an open reading frame in which the first codon spans from nucleotide 10 through nucleotide 12 of SEQ ID NO:119, and the stop codon spans from nucleotide 442 through nucleotide 444 of SEQ ID NO:121. The encoded protein has a predicted molecular weight of 16 kDa. The coding region encoding $PFeGMCSF_{144}$ is presented herein as $nFeGMCSF_{432}$ which has the nucleotide sequence SEQ ID NO:122 (the coding strand) and SEQ ID NO:123 (the complementary strand). A putative signal peptide cleavage site is between amino acid positions 17 and 18, based on homology with human, mouse and ovine GMCSF proteins. The nucleic acid molecule encoding the proposed mature protein is denoted as $nFeGMCSF_{381}$ and has a nucleotide sequence represented herein as SEQ ID NO:124 and a complementary sequence represented herein as SEQ ID NO:126. The amino acid sequence of the putative mature protein, referred to herein as $PFeGMCSF_{127}$, has an amino acid sequence represented herein as SEQ ID NO:125. The number of amino acids in the feline GMCSF protein is the same compared to human, porcine, ovine and canine GMCSF proteins. The feline GMCSF protein is one amino acid longer than bovine GMCSF and three amino acid longer than murine GMCSF.

The deduced amino acid sequence of the full-length feline GMCSF protein of the present invention has four non-conservative changes and one conservative change compared to a GenBank entry for feline GMCSF (accession # AF053007). Amino acids

asparagine, methionine, threonine, and lysine at positions 10, 36, 56 and 126 of the GenBank entry have been changed to glycine, isoluecine, alanine and asparagine, respectively, in $PfeGMCSF_{144}$. $PFeGMCSF_{144}$, containing the above-noted amino acid substitutions, appears to have GMCSF activity, as demonstrated by an experiment in which supernatant collected from Chinese Hamster Ovary (CHO) cells that were transiently transfected with a recombinant molecule encoding a feline GMCSF protein of the present invention was able to induce proliferation of TF-1 cells.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.